Plant Resource Allocation

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The Fate of Acquired Carbon in Plants: Chemical Composition and Construction Costs

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I. Introduction

One of the goals of ecophysiology and agronomy is to understand the physiological basis of plant growth. In this respect, the process of photosynthesis has received ample attention. Large efforts have been made to analyze the physical and biochemical processes that are necessary for carbon fixation. The subsequent fate of the acquired carbon (C) has been investigated far less. Factors controlling the plant's respiration and translocation of C to several organs are only fragmentarily understood. This applies even more so to the fate of C in a specific organ or cell. In most studies related to plant growth, biomass accumulation is taken as such and not analyzed further. However, the way a plant invests its carbon as well as the other acquired elements into different compounds may have a profound effect on its growth and performance in a certain environment (e.g., Bazzaz *et al.,* 1987). Therefore, it is crucial to understand the factors controlling chemical composition.

So far, we only have a limited understanding of the causes and consequences of variation in chemical composition. In this chapter, we discuss first the integration level at which the chemical composition of plants will be considered. Second, we characterize the chemical composition of various plant organs at that integration level. What values for the concentrations of the various plant compounds could be taken as typical for an "average"

plant, and what variation is to be expected around these average values? Third, we analyze to what extent these compounds covary in specific patterns. That is, in comparing different species or a range of environments, will a rise in compound A always be accompanied by a decrease in compound B? Or do both compounds vary independently? Fourth, we review the possible mechanistic explanations that have been put forward to explain variation in chemical composition.

The last two sections of this chapter discuss the consequences of variation in chemical composition. Given the chemical composition of a plant, it is possible to arrive at an estimate of the total amount of photosynthate that has to be spent to construct one gram of biomass: the so-called construction costs. First, we focus on these construction costs, and discuss to what extent they depend on environment and type of species. Second, we briefly discuss the ecological consequences of variation in chemical composition.

II. Integration Level

Plants contain a vast range of compounds, with estimates of more than 100,000 present (Buckingham, 1993), most of them in very small amounts. A complete analysis of all these compounds is impossible. Although powerful techniques are available now to analyze small samples on large ranges of constituents (e.g., pyrolysis-mass spectrometry; Boon, 1989), such a detailed picture would not be of much help to understand processes at a higher integration level. Rather, it is preferable to categorize these compounds in a limited number of classes of constituents, which yields the "proximate" chemical composition (cf. Penning de Vries *et al.,* 1974). In this chapter we use eight different categories: (1) lipids, (2) lignin, (3) soluble phenolics (tannins, flavonoids), (4) organic N compounds (which we will call "protein" troughout this chapter but which consist of at least DNA, RNA, chlorophyll, and amino acids as well), (5) total structural carbohydrates (TSC: cellulose, hemicellulose, and pectin), (6) total nonstructural carbohydrates (TNC: starch, fructan, sucrose, fructose, glucose), (7) organic acids, and (8) minerals. In general, these compounds together comprise more than 90-95% of a plant's biomass (Chapin, 1989; Poorter and Bergkotte, 1992). The other constituents of a plant are mostly present in only small concentrations. However, in some species compounds like cyanogenic glucosides (Merino *et al.,* 1984) or terpenes (Bryant *et al.,* 1983) are found in relatively large amounts (>5%) of the plant's biomass. This classification is very broad and so may not be suitable for some kinds of ecophysiological problems. For example, in cases where water relations are of interest, it may be useful to separate the soluble fraction from the insoluble part of the TNC, and combine the soluble sugars with the organic acids, as both have a similar osmotic function.

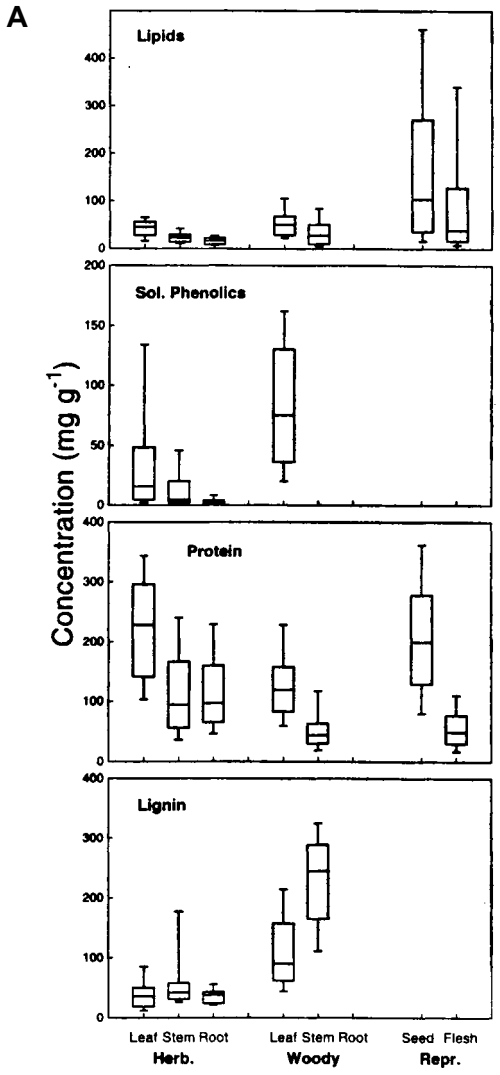
Generally, not all of the eight groups of compounds are determined on the same biological samples. Although this is not always necessary to answer a specific question, there is an added value in knowing the overall proximate chemical composition of the various organs of a plant. In this way, tradeoffs between allocation of C and nutrients to constituents with different functions in the plant can be evaluated.

Concentrations of chemical compounds in plants can be expressed in various ways. In the literature, values on either a fresh or dry weight basis are used, per unit of leaf area or as a ratio, relative to another compound. Moreover, concentrations are analyzed for integration levels varying from organelles up to that of the whole plant. In this chapter we follow the majority of papers and express concentrations per unit dry weight. This avoids the problem of variation in dry weight: fresh weight ratios or leaf area: leaf weight ratios that could mask genuine differences in concentrations. However, depending on the context of the research, other expressions may be more useful in specific cases. Concerning the integration level within the plant we differentiate between leaves, stems, roots, as far as vegetative organs are concerned, and seeds and fruit flesh as the two major components of reproductive organs. We believe that concentrations expressed on a whole plant weight basis may be helpful as well in understanding the growth and functioning of plants (see Poorter, 1994). However, due to differences in composition between organs, concentrations per unit total plant are confounded with the allocation pattern of biomass to the different organs, and we will not use it here.

In Appendix 1, a procedure for a proximate analysis is presented, as used by one of us (Poorter and Bergkotte, 1992). Reference is made to the various determinations necessary. Appendix 2 lists the subsequent assumptions and calculations to arrive at an estimate of the different classes of compounds. Another scheme for a more or less complete extraction is given by Kedrowski (1983).

III. Chemical Composition

Before being able to discriminate between "low" and "high" values for the different constituents, we have to establish "normal" concentrations and their variation. Only a few attempts have been made to fully characterize the proximate chemical composition of a plant. These do not suffice to obtain a general picture. Therefore, we screened the literature for determinations of each of the eight groups, compiling data from a wide variety of sources. Median values, as well as the ranges generally found, are given in



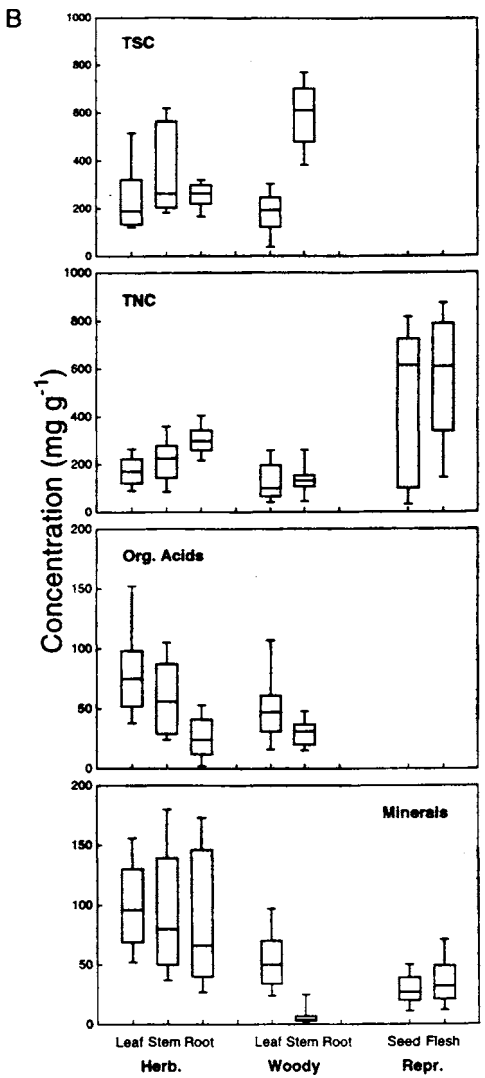


Figure 1 Characterization by box plots of the chemical composition of various plant tissues for (A) relatively expensive classes of compounds and (B) relatively inexpensive classes of compounds. All concentrations are expressed in milligrams per gram dry weight. Data are categorized into values pertaining to herbaceous species (leaf, stem, root), woody species (leaf, stem, root), and categories of seeds and fruit flesh. Values are extracted from a wide range of literature sources (among others. Bliss, 1962; Loveless, 1962; Gaspers, 1977; Duke and Atchley, 1986; Chapin, 1989; Fengel and Wegener, 1989; Poorter and Bergkotte, 1992; Jordano, 1995). No data are presented in categories in which we collected less than 20 observations. The number of observations on concentrations in leaf, stem, and roots of herbaceous plants and leaves, stems, and roots of woody species, as well as for seeds and fruit flesh, are as follows: lipids, 190,50,80/200,60,—/320,490; lignin, 80,40,30/100, 180,—/—,—; soluble phenolics, 70,40,30/110,—,—/—,—; protein, 360,150,160/260,90,—/ 340,560; TSC, 100,50,30/80,90,—/—,—; TNC, 190,50,100/80,20,—/60,460; organic acids, 130,30,30/30,20,—/—,—; minerals: 420,50,90/250,160,—/320,260. Box plots indicate the distribution by percentiles. The xth percentile is the value below which *x*% of the observations are found. The lower and higher part of the box indicate the 25th and 75th percentiles, respectively. The value of the error bars are the 10th and 90th percentile, and the 50th percentile (median) is given by the horizontal line within the box.

Fig. 1. It should be stressed that the compiled values were obtained with different methods of quantification, for plants grown under a wide range of conditions. Especially the concentrations of protein, TNC, and minerals will depend strongly on the levels of resource supply (Waring *et al.,* 1985; McDonald *et al.,* 1986; Griffin *et al.,* 1993). Nevertheless, Fig. 1 provides a useful indication of the concentrations that are to be expected. In leaves of herbaceous species, median values are highest for protein followed by TSC, TNC, and minerals, each of which comprise 10% or more of the leaves' biomass. Organic acids, lipids, and lignin show median concentrations around 5%, and levels of soluble phenolics are just a few percent. Stems and roots of herbaceous plants have lower concentrations of protein, organic acids, soluble phenolics, and lipids, and higher values for TSC and TNC than leaves. Compared to leaves of herbaceous species, leaves of woody species contain less proteins, minerals, and organic acids, similar amounts of lipids, TNC, and TSC, and higher concentrations of lignin and soluble phenolics. As far as information is available, the same pattern is found when stems from herbaceous species are compared with those of woody species, with the notable exception of TSC, which shows much higher concentrations in stems of woody plants. We were not able to find enough data for a reliable estimate of the chemical compositions of roots of woody species. Judging from available information, they are quite similar to those of woody stems. On average, seeds and fruit flesh are characterized by rather high concentrations of TNC and lipids, and lower concentrations of minerals. A difference between the two is that seeds can have high concentrations of protein, whereas this is not the case in fruit flesh. Variation in chemical composition of the reproductive plant parts is much larger than in vegetative organs. This obviously reflects the function of these plant parts in accumulating storage compounds of various types, or attracting different animal seed dispersers (see Jordano, 1995).

In the above compilation, data from a wide range of species and growth conditions were combined. To what extent do specific investigations on differences between organs or species support the above trends? Poorter and Bergkotte (1992) analyzed the levels of the eight classes of compounds described above for leaves, stems, and roots of 24 herbaceous species. For each species, the concentration in stems, and roots was calculated relative to that in the leaves. Subsequently, for an overall impression these values were averaged over all species investigated (Table I). In both stems and roots, concentrations of lipids, soluble phenolics, protein, and organic acids were lower, whereas those of lignin, TSC, TNC, and minerals were higher than in leaves. As far as determined, these observations are in line with those of Niemann *et al.* (1993) on tomato and of Challa (1976) on cucumber. We are not aware of data on whole stems and roots of woody species. An interesting aspect shown in the data of Table I is that differences in chemical

Table I Ratios of Concentrations of Compounds in Stems and Roots, Relative to Those of Leaves[a]

Compound	Stem	Roots
Lipids	0.57***	0.49***
Organic acids	0.77**	0.28***
Soluble phenolics	0.77*	0.64**
Protein	0.80***	0.70***
Minerals	1.27***	1.46***
TSC	1.39***	1.69***
Lignin	1.78***	1.69***
TNC	1.85***	2.15***

[a]Average values of data on 24 herbaceous species (Poorter and Bergkotte, 1992). Values are back-transformed averages after natural log transformation of the ratios, to correct for the ln-normal distribution of ratios. Asterisks indicate to what extent the average deviates from 1.0 (H_0 hypothesis, no difference between leaves and stems or roots): *, $P < 0.05$: **, $P < 0.01$; ***, $P < 0.001$.

composition between stems and roots are small, when they are compared with those of leaves. Clearly, if one prefers to analyze plant composition in a two- rather than three-compartment model, it is advisable to separate leaves from stems and roots, rather than separating aboveground and belowground plant parts.

Although there is common knowledge about how groups of species differ in composition, we are not aware of many larger scale comparisons to support these notions with a quantitative basis. Poorter and Bergkotte (1992) and Niemann *et al.* (1992) found higher concentrations of protein, organic acids, and minerals in potentially fast-growing species, and higher concentrations of cell-wall compounds in herbaceous species with a low growth potential (see also Section IV). In a comparison of four woody and six herbaceous species, grown at a nonlimiting nutrient supply, a clear difference was found in the concentration of minerals (H. Poorter and J. R. Evans, unpublished). In particular, NO_3^- was present in small concentrations in the leaves only of woody plants. Differences in organic acids and proteins showed similar directions as those in Fig. 1, although the differences were rather small. The more clear-cut differences in Fig. 1 may be a reflection of the fact that most data on woody species are from material collected in the field. Within the group of the woody plants, evergreen and deciduous species differ in their chemical composition. Leaves of deciduous plants have higher concentrations of proteins (Loveless, 1962) and minerals than leaves of evergreens (R. Villar and J. Merino, unpublished). The concentration of lipids were found to be similar.

Environmental impact on chemical composition is especially large for protein, lignin, minerals, and TNC. High-light conditions result in high concentrations of lignin, whereas low-light plants accumulate more minerals (Waring *et al.,* 1985; H. Poorter and J. R. Evans, unpublished). Plants grown at high nutrient levels have higher concentrations of protein, and minerals and lower levels of TNC (Waring *et al.,*1985; McDonald *et al.,* 1986). Plants at high CO_2 accumulate TNC to a large extent, and apart from this show lower protein and mineral concentrations. Lignin concentrations are hardly affected (Körner *et al.,* 1995; Poorter *et al.* 1997).

IV. Covariation in Plant Compounds

Given the ranges that can be expected in the various compounds (Fig. 1), the next question to address is to what extent compounds covary. That is, will high values of constituent A always be accompanied by a high (or a low) concentration of constituent B? Insight into these patterns is required before a proper analysis of the regulation of chemical composition can be made. This question about covariation can be answered at two levels, a methodological and a biological one. Concentrations of compounds are generally expressed per unit total dry weight Therefore, in the end the total composition will always add up to a concentration of 100%. Consequently, it would be expected that different plant compounds covary in a negative way, due to a change in one compound only. For example, an increase in the concentration of compound A from 100 to 250 mg g^{-1} will automatically imply a decrease in all other compounds of 13%. As there is no "internal standard" to which to relate changes in composition, there is no straightforward solution to this problem. In cases where large absolute changes are to be expected, like the accumulation of starch in the leaves of plants grown at elevated CO_2 (e.g., Körner *et al.,* 1995), it may be worth considering concentrations on a TNC-free basis. Alternatively, concentrations have been expressed per unit fresh weight. As the dry biomass generally comprises just a small part of the total fresh weight, changes in one compound will only have a small effect on changes in another. However, with such a solution it is implicitly assumed that there is hardly any difference in the water content per unit dry weight. Any variation in water content between organs or species will confound the results.

Apart from the methodological, negative correlation mentioned above, covariation can have a biological background. For example, if plants are grown at a range of N availabilities, there is generally a negative correlation between the amount of protein and TNC, even if the amount of protein

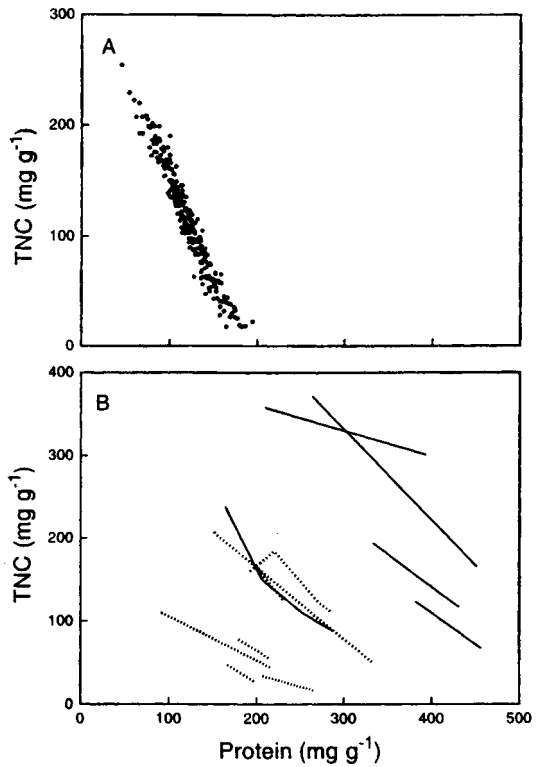


Figure 2 Relationship between the amount of TNC (or starch) and protein (on a TNC-free basis) in leaves or shoots of various plant species. (A) Two thousand observations on *Oryza sativa* (Batten *et al.,* 1993). All values were ranked on the basis of their TNC value. Data points give the average values for 10 consecutive individuals in the ranking. (B) Data on several herbaceous (continuous lines) and woody (broken lines) species, grown at various levels of N availability. Data are from Hehl and Mengel (1972), Waring *et al.* (1985), McDonald *et al.* (1986), Landsberg (1987), Wong *et al.* (1992), and Mooney *et al.* (1995).

is expressed on a TNC-free basis (Fig. 2). This is an intriguing phenomenon, which is not well understood. Given the high concentration of nonstructural carbohydrates in the leaves at low N availability, we can deduce that the amount of photosynthates fixed during photosynthesis is in itself not the factor limiting growth of these plants. Clearly, low-N plants have an excess of sugars. If it is not the amount of photosynthetic machinery that limits the growth at N limitation, it must be the amount of N invested in nonphotosynthetic compounds. Apparently, at a low nitrogen availability, plants over-

invest N in compounds related to the photosynthetic machinery. If we would assume that plants try to maximize their growth in a given set of limiting resources, it has to be concluded that these low-N plants generally behave suboptimally under such conditions. As yet, we have no idea about the reason for this behavior.

Covariation in chemical composition is not only environmentally induced, but could also be due to inherent differences between species. As noted above, even for different species grown under the same conditions, variation in chemical composition is considerable. Poorter and Bergkotte (1992) analyzed the chemical composition of leaves, stems, and roots of 24 wild herbaceous species, differing in potential relative growth rate. How do the different compounds covary in these cases? To investigate this we carried out a factor analysis (Fig. 3). This is a way to characterize how well related a range of variables is (see the legend to Fig. 3 for an explanation). As can be seen, there is a general clustering of cytoplasmic and vacuolar compounds (protein, minerals, organic acids) on one hand (see Poorter, 1994) and cell-wall compounds (lignin, TSC) on the other hand. This is true for all the organs of these species (Fig. 3A,C,D). Positively correlated with the cytoplasmic/vacuolar complex are the total N concentration, the potential growth rate of the investigated species, the leaf area: leaf dry weight ratio (specific leaf area, SLA), as well as the water content per unit dry weight. Positively correlated with the cell-wall complex is the C concentration.

How general are the observed trends? Do they still hold when species of different life forms are compared? Poorter *et al.* (1997) analyzed the proximate chemical composition of leaves of 7 trees, 9 crop species, and 11 wild herbaceous plants. Only three of these were used in the previous experiment as well. All species were grown under controlled conditions with a relatively high nutrient supply, but in different laboratories. Therefore, light conditions, temperature, and soil substrate may have varied to some extent. It is striking how similar the results (Fig. 3B) are when these are compared with the leaves of the 24 herbaceous species (Fig. 3A), with again a clustering of protein, minerals, and organic acids on the one hand, and lignin and soluble phenolics on the other. The exceptions are the lipids and TSC, which have swapped places. The strong correlation of lipids with the "slow-growth/low SLA" complex is due to the evergreen *Eucalyptus* species within the data set, which showed higher concentrations of lipids (50-100 mg g^{-1}) than the herbaceous species. Relatively high values of lipids in leaves of evergreen species as compared to deciduous shrubs and trees have also been found in a large survey of field-grown species (R. Villar and J. Merino, unpublished). That survey showed a positive correlation between protein and minerals within the woody species as well. We are not aware of many experiments where a wide range of parameters have been

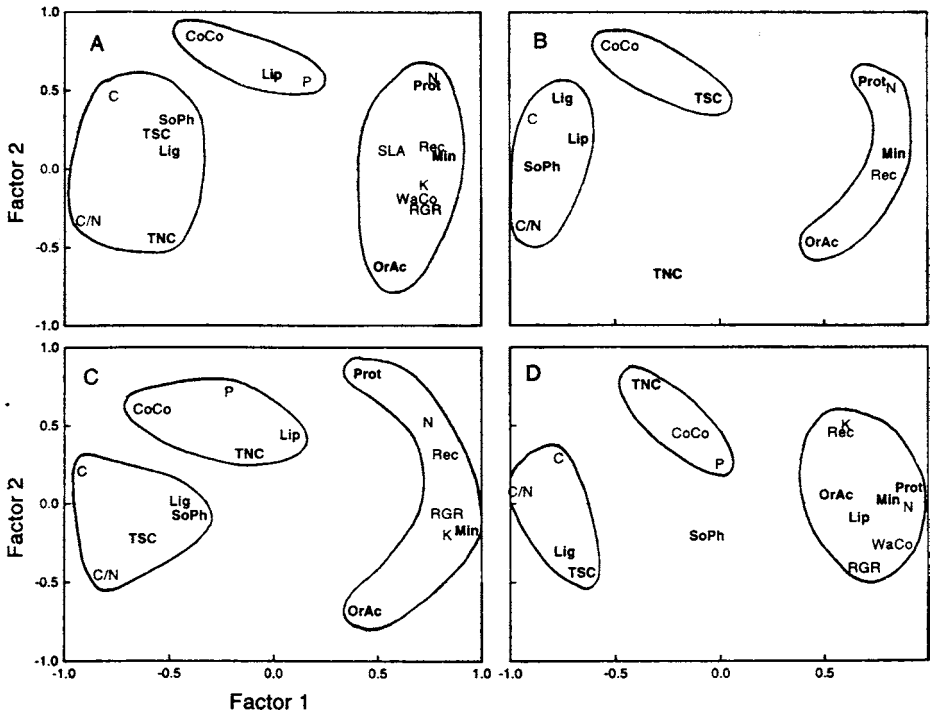


Figure 3 Principal component analysis of data on the chemical composition of a range of plant species. (A) Leaves of 24 different herbaceous plant species, varying in relative growth rate (RGR). RGR, specific leaf area (SLA, leaf area/leaf dry weight), and water content (g water/g dry weight) of the leaves are included as well. (B) Leaves of 27 species (7 woody, 9 crop species, and 11 herbaceous wild plants), all grown at high nutrient availability. (C) Stems and (D) roots of 24 different herbaceous plants, varying in potential relative growth rate. RGR and water content of the stems and roots, respectively, are included as well. The two factors explained 50-60% of the total variation. Data of A, C, and D are from Poorter and Bergkotte (1992), and data of B are from Poorter *et al.* (1997). Abbreviations: CoCo, construction costs; Lig, lignin; Lip, lipids; Min, minerals; OrAc, organic acids; Prot, protein; Rec, recovery (total fraction of the biomass explained by the sum of all concentrations); SoPh, soluble phenolics; TSC, total structural carbohydrates; TNC, total nonstructural carbohydrates; WaCo, water content. Abbreviations in bold type pertain to the eight classes of compounds indicated in Section II. In this analysis two new variables (factor 1 and factor 2) are computed out of a combination of all original variables. For each of these variables it is calculated whether they contribute positively (close to 1.0), negatively (close to -1.0), or not (close to 0.0) to factor 1. The amount of variance thus explained is taken out of the data, and the procedure is repeated with the remaining variance. The result is somewhat comparable to a two-dimensional electrophoresis. Variables that are close together (like total N and protein in Fig. 3B) are generally positively correlated, variables that are at opposite parts of the graph (like minerals and soluble phenolics) are negatively correlated, and variables that have values close to 1 or -1 for one factor and values close to 0 for the other axis (like minerals and, to some extent, TSC) are generally not correlated at all.

determined simultaneously. Dijkstra (1989) found similar correlation patterns as in Fig. 3 for two *Plantago major* subspecies, differing in relative growth rate (RGR), SLA, leaf water content, TNC, minerals, and total cell-wall material. At variance with the trends observed here, protein concentration did not differ for these subspecies.

It is noteworthy that these patterns of investment, either with high concentrations of proteins, minerals, and organic acids or high concentrations of lignin and cell-wall components, coincide with the concentration of total C and N, and with the C/N ratio. By definition, C is positively and N negatively correlated with the C/N ratio (cf. panels in Fig. 3). However, as variation in the C concentration is generally confined to the range 400-500 mg g^{-1}, whereas N may vary more than fivefold, C/N ratios mainly depend on variation in N. Lignin and TSC generally cluster together with C and the C/N ratio, but vary more than C (Fig. 3). This supports the use of parameters like the crude fiber:protein ratio (Loveless, 1962) or lignin: N as applied in decomposition studies (Berg and Staaf, 1980; Melillo *et al.,* 1982). However, if these parameters are not available, C/N ratios are useful descriptors of plant material. An advantage of die use of the C/N ratio is that it can be determined easily with an elemental analyzer, independently of extractions and colorimetric or enzymatic reactions. Thus, interactions of the determination with other compounds, which generally result in lower estimates, do not occur. Such drawbacks are characteristic of the usual proximate analyses (see Poorter, 1994). Note, for example, that the sum of the amount of dry weight ascribed to the various classes of compounds (indicated as "recovery") is correlated positively with RGR and water content, but negatively with investment in cell walls (Fig. 3). This could be due to the fact that slow-growing species do have compounds we did not analyze for (like the terpenes mentioned above). Alternatively, and most likely, the chemical assays may have been disturbed by the wealth of secondary compounds in the plant material.

V. Mechanistic Explanations for Variation in Chemical Composition

What is the reason for the emerging patterns of compounds within a given organ? Which physiological and/or morphological characteristics determine whether a plant will have a low or a high concentration of a given constituent? The underlying mechanisms are not well understood. In this section, we discuss a number of possible explanations. In some cases we are only able to correlate chemical composition with some other plant traits, without understanding the mechanisms behind it. In searching for explanations it is necessary to partly deviate from the distinction of the

eight categories we have made, and to consider specific compounds and processes at a more detailed level.

A first explanation for the negative correlation between protein and minerals, on the one hand, and cell walls, on the other, may simply be ontogeny (Chapin, 1989). Young material, just formed, will have high concentrations of protein, whereas older tissue has undergone deposition of secondary cell walls and will show higher concentrations of lignin and TSC. If fast-growing species had relatively large amounts of young tissue, as compared to slower growing plants, this might explain the negative correlation.

A second cause for the distinction between species investing in cytoplasmic/vacuolar compounds and those investing in cell-wall compounds may be a difference in anatomy at the cellular level. If large numbers of sclerenchyma cells are formed, this will obviously increase the amount of lignin and TSC relative to that of protein (see Gamier and Laurent, 1994; Van Arendonk and Poorter, 1994). In addition, relatively high proportions of cell-wall compounds are also expected in small-sized cells with a high cell wall area:cell volume ratio.

To some extent, differences in the concentration of starch between species are correlated with the mode of phloem loading. That is, species that have a symplastic type of phloem loading have much higher starch concentrations in the leaves than species that load apoplastically (Van Bel, 1994; Körner *et al.,* 1995). The cause for the difference in TNC accumulation between the two groups has not yet been clarified.

It has been suggested that lignification could hinder a high metabolic activity, due to a low water permeability of the cell wall, and that this would be a reason for high-protein plants having low concentrations of lignin (Chapin, 1989). We consider this explanation to be unlikely. Lignin is generally accumulated in cells that are dead, or at best have a marginal metabolic activity (sclerenchyma, tracheids, xylem vessels). Cells with a high metabolic activity (palisade and spongy parenchyma, accompanying cells) do not show significant lignification.

Other explanations for the observed patterns are at the biochemical/physiological level. A clear cause and effect can explain the positive correlation between the concentrations of protein and organic acids in the leaf. According to the Benzioni-Lips model, NO_3^- which is transported to the leaf is reduced there. The negative charge of the nitrate is transferred to an organic acid (malate), which will partly be transported to the roots and broken down, and partly accumulated in the vacuoles of leaf cells (Dijkshoorn *et al.,* 1968). Thus, a rather close correlation between the concentrations of organic acid and protein is expected. Indeed, both load high on the first axis in the factor analysis (Fig. 3A,B). However, it is noteworthy that in both screening experiments there is a separation be-

tween protein and organic acids, when the second factor is considered. We have no explanation for this pattern, which is observed in stems as well (Fig. 3C). A close correlation between leaf protein and organic acids is not expected either in plants that predominantly reduce nitrate in the root, such as tree species (Gojon *et al.,* 1994), or in leaves of plants grown with ammonium (Dijkshoorn *et al.,* 1968; Raven, 1985).

Apart from the differences due to inherent variation in leaf anatomy, the relative investment in cytoplasmic/vacuolar compounds versus cell-wall compounds may also be regulated at the biochemical level. Two major theories have been put forward. In the first, synthesis of quantitative secondary compounds is thought to be regulated by the amount of sugars available. In the case of, for example, low nutrient availability, but also for inherently slow-growing species, total nonstructural carbohydrates accumulate (Figs. 2 and 3), which may trigger incorporation of C in carbon-based compounds (Bryant *et al.,* 1983), largely invested in cell walls. Alternatively, and more specifically focused on compounds of a phenolic nature, there may be some kind of competition between synthesis of proteins and that of phenolics. In both pathways the amino acids tyrosine and phenylanaline play a role. At high levels of protein production, the amino acids would be readily incorporated into proteins. If protein synthesis is low, however, phenolics may be produced (Margna 1977). Lambers (1993) reviews both theories and concludes that the experimental evidence up to now is mainly correlative.

Fast-growing species have higher amounts of water per unit dry weight, and therefore require relatively large amounts of osmotics. Consequently, high concentrations of minerals, soluble sugars, and/or organic acids per unit dry weight are found (see Fig. 3). The exact osmotic used may be a regulation point by itself. For example, Blom-Zandstra *et al.* (1988) found that one genotype of *Lactuca sativa* accumulates relatively high concentrations of organic acids in its vacuoles, whereas another mainly uses nitrate. The latter was growing fastest, possibly reflecting the fact that a larger part of the fixed C could be invested in structural growth (see Raven, 1985). Similarly, there could be a regulation mechanism depending on light intensity: the lower the light intensity, the more of the osmotically active sugars are replaced by nitrate (Wedler, 1980).

Candidates for a possible regulatory role are the hormones. However, we are not aware of many studies that have investigated this aspect. Niemann *et al.* (1993) studied the chemical composition of wild-type tomatoes and mutants deficient in gibberellic acid (GA). The mutants had higher concentrations of protein and lower concentrations of cellulose. This might be related to the relatively larger cells of the GA-deficient mutants. These changes may therefore be a rather indirect effect of hormone production.

VI. Construction Costs

A. Carbon Budget

What are the consequences of differences in chemical composition for plant growth? There are two ways to approach this problem. First, one can construct a carbon budget. Such a budget relates quantitatively to the relative growth rate of a plant:

$$RGR = \frac{PS_a \times SLA \times LWR - LR_w \times LWR - SR_w \times SWR - RR_w \times RWR}{C_L \times LWR + C_S \times SWR + C_R \times RWR}, \quad (1)$$

where PS_a is the rate of photosynthesis per unit leaf area and LR_w, SR_w, and RR_w are the rate of respiration per unit leaf, stem, and root weight, respectively. All these values are expressed as fluxes of C integrated over a day. LWR, SWR, and RWR are the fractions of biomass allocated to leaves, stems, and roots, respectively, SLA is the specific leaf area, and C_L, C_s, and C_R are the concentrations of carbon per unit dry weight of the three organs. The total carbon gain of a plant, expressed per unit total plant weight and integrated over a day, is represented by Fig. 4A. A fraction of the C fixed over that day is respired again. This respiration is used to provide energy and/or reducing power for maintenance, to take up minerals by the roots, for transport of compounds, as well as for growth (Van der Werf *et al.,* 1994). The remaining fraction is considered to be C invested in new growth as C skeletons. However, the actual amount of biomass which can be formed

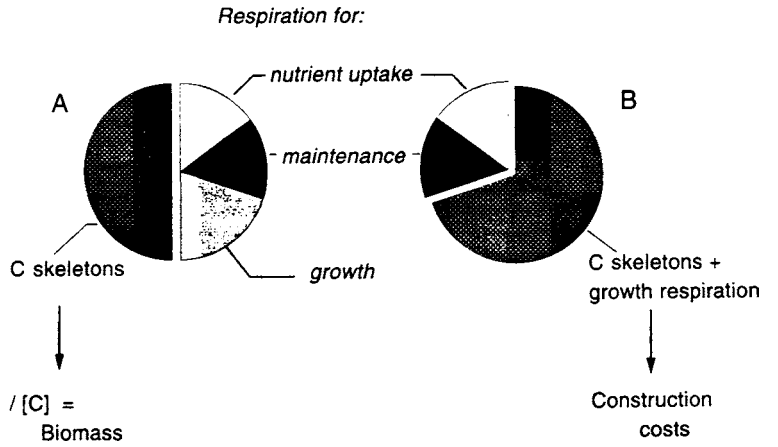


Figure 4 Representation of the total carbon gain and the fate of the fixed glucose. (A) Separation into glucose C spent in respiration and C invested in C skeletons. (B) Separation into glucose C spent in respiration for maintenance and uptake of nutrients, and construction costs (growth respiration plus C skeletons).

will depend on the carbon concentration of that biomass. Generally, the C concentration varies from 400 mg g^{-1} in herbaceous plants grown hydroponically up to 550 mg g^{-1} in highly lignified trees (Poorter, 1989). Consequently, everything else being equal, it can be derived from Eq. (1) that relative growth rate may vary by 38% due to variation in the amount of C that is present in 1 gram of biomass.

A second avenue to analyze the growth of plants is to split up the C-requiring processes in a slightly different way, by adding the C spent in growth respiration to the C invested in C skeletons (Fig. 4B). In this way, we arrive at the construction costs of a plant, which are defined as the amount of glucose required to construct 1 gram of biomass (Penning de Vries *et al.,* 1974; Williams *et al.,* 1987). Consequently, this value not only includes the glucose for providing C skeletons, but also the NAD(P)H and ATP to drive the energy-requiring reactions (Fig. 4).

How do construction costs relate to the chemical composition, as discussed in the previous sections? Taking the most likely biosynthetic pathways, the amount of glucose required to build 1 gram of any of the eight classes of compounds can be calculated (Penning de Vries *et al*., 1974; Lambers and Rychter, 1989). These values, which include the reducing power and ATP necessary, are listed in Table II. The costs of uptake and transport are accounted for in Fig. 4 by respiration for uptake. Consequently, the construction costs for minerals are nil. Within the group of organic compounds, differences amounting to a factor of 3 are present. Clearly, the construction costs of the plant will depend on the relative contribution of expensive compounds, like lipids, lignin, soluble phenolics, and protein, on the one hand, and the level of cheap compounds, like TSC, TNC, organic acids, and minerals, on the other.

Table II Amount of Glucose Required for and Amount of CO_2 Produced during Synthesis of 1 Gram of Different Compounds[a]

Compound	Construction costs (g glucose g^{-1})	CO_2 produced (mmol g^{-1})
Lipids	3.03	36.5
Soluble phenolics	2.60	31.9
Protein (with NO_3^-)	2.48	37.9
Lignin	2.12	13.1
TSC	1.22	2.8
TNC	1.09	1.8
Organic acids	0.91	-1.0
Minerals	0	0

[a] Data from Penning de Vries *et al.* (1983) and Lambers and Rychter (1989).

B. Limitations

There are a number of technical as well as conceptual limitations related to the determination and use of construction costs in ecophysiological research. Technical details and assumptions are discussed by Chiariello *et al.* (1989) and Poorter (1994). Here we concentrate on the conceptual issues. First, the costs of the various compounds are based on the most likely biochemical pathways. It is not necessarily true that biosynthesis follows these routes. We are not aware of a systematic evaluation of possible errors involved. Penning de Vries *et al.* (1974) assessed variation in construction costs for different constituents within several of the classes of compounds distinguished here. Differences were generally within the 5% range and were small compared to differences between classes of compounds.

Second, it is assumed that the respiration involved in growth processes has a P/O ratio of 3. That is, when respiration is running most efficiently, three molecules of ATP are formed per oxygen atom (O) consumed. However, if the alternative pathway of respiration is operational, the P/O ratio will decrease. The alternative pathway of respiration yields only one ATP per O consumed (Lambers, 1985). If the alternative pathway respiration represents half of the total respiration rate, the P/O ratio will decrease to about 2. Consequently, more glucose is required to provide for the accessory energy. The exact amount of extra glucose depends on the chemical composition of the plant. For the range of woody, wild herbaceous, and crop species from Fig. 3B, the change in P/O ratio from 3 to 2 would lead to a 4.5-6% increase in construction costs (see Lambers *et al.,* 1983; Amthor, 1989).

Third, there is the problem of the exact N source of the plant. If N is taken up in the form of NH_4^+ exclusively, the plant does not need to provide energy for the reduction step from NO_3^- to NH_4^+. If N is taken up as NO_3^- and reduced in roots or stems, these costs must be included. When a plant uses NO_3^- and reduces this in the leaves, matters are more complicated. Part of the reduction, from nitrite to ammonium, takes place in the chloroplasts, and the reducing power necessary for the reduction could be drawn directly from the NADPH supply by the light reaction (Layzell, 1990). In this way, the steps of converting CO_2 to glucose and respiring it thereafter are avoided. Consequently, these costs do not need to be included in the carbon budget. Most of the studies on construction cost consider NO_3^- as the nitrogen source and assume that nitrate reduction comes at full cost. This will therefore yield maximum values for the construction costs. For a plant with a leaf protein concentration of 230 mg g^{-1}, the difference in construction costs, with NO_3^- or NH_4^+ as nitrogen source, would be 0.20 g glucose g^{-1}. This could represent about 13% of the construction cost. For stems and roots, which have much lower protein concentra-

tions (Fig. 1A), this value will be less. An estimate of the form of N taken up by the plant could be obtained by assessing the difference between cations and anions, relative to the amount of organic N in the total plant (Troelstra, 1983).

Given the assumptions stated above, one may question how well these construction costs are estimated. Not many independent checks have been made. Poorter (1994) determined photosynthesis, respiration, biomass allocation, and chemical composition of a range of herbaceous species, differing in potential RGR by 300%. He calculated the construction costs per species from the chemical composition. With a slight alteration of Eq. (1) he could estimate RGR from these parameters as follows:

$$RGR = \frac{PS_A \times SLA \times LWR - LR'_w \times LWR - SR'_w \times SWR - RR'_w \times RWR}{CC \times \frac{6}{180}}, \quad (2)$$

where LR'_w and SR'_w are the maintenance respiration rate for leaves and stem, respectively, RR'_w the respiration rate of the roots related to maintenance and uptake of nutrients, and 6/180 is the multiplication factor to convert whole plant construction costs (CC) from grams of glucose to moles of C. Maintenance respiration is defined as the respiration an organ needs to supply the energy for keeping the organ functioning. The RGR values for each species calculated in this way were lower than those determined experimentally. Apparently, the costs in terms of CO_2 losses were overestimated to some extent. Given the uncertainties around each of the determinations, this could just be a matter of chance. However, if the assumption that the plants reduced nitrate at the cost of glucose breakdown was lifted, estimated and determined values agreed quite well. This in itself would lend support to the idea that nitrite reduction takes place largely in the chloroplasts, and could consume reducing power which would otherwise not be used for C fixation (Layzell, 1990).

Verification of the construction cost values could proceed at a lower integration level as well. The amount of carbon required for the carbon skeletons of the plant material can be determined precisely. Given a C concentration in plant material of 400 mg g^{-1}, the amount of glucose required to provide the C skeletons for 1 gram of biomass is 1000 mg. The other part of the construction cost is the glucose, which will be respired to provide for the NADPH and ATP required for biosynthesis. This is the so-called growth coefficient, which we express here as the amount of glucose respired to synthesize 1 gram of biomass. It is this part that needs independent confirmation. Plant respiration can be measured relatively easily, but it will include respiration involved in both growth and maintenance. One of the most commonly used methods to separate these two is the regression method developed by Thornley (1970). In this method, total respiration per unit organ weight and time *(R)* is written:

$$R = m + g \times RGR, \quad (3)$$

where *m* and *g* are the maintenance and growth respiration, respectively, and RGR the specific growth rate of the organ or plant. How well do these independent estimates of *g* match the amount of glucose that is calculated to form the part of the construction cost which is to be respired? We compiled both *g* and construction cost values of the literature. We assumed herbaceous and woody plants to have a C concentration of 400 and 450 mg g^{-1}, respectively. By deducting the glucose necessary for C skeletons (1.0 and 1.125 g glucose g^{-1}, respectively, for the two groups of species) from each of the total estimated construction cost values, a second estimate of *g* was obtained. Somewhat as a surprise, these two independent estimates showed almost exactly the same range and distribution (Fig. 5). This gives some credibility to the approaches used, although we consider the proof still not good enough. Estimates where construction costs and *g* are determined

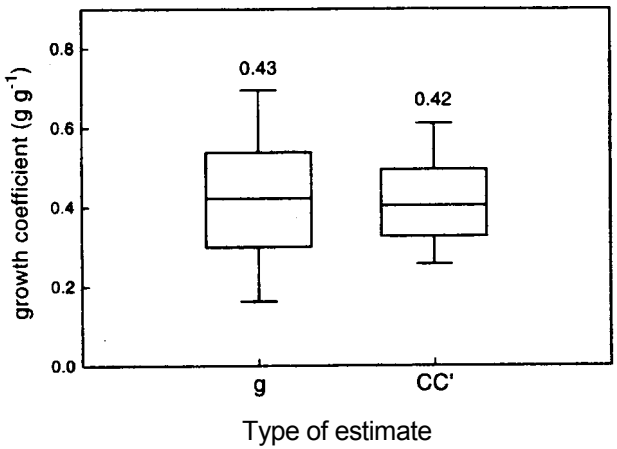


Figure 5 Characterization of the distribution of the growth coefficient *g,* the respiration required for the construction of 1 gram of biomass, as derived from two independent estimates. In the first approach, *g* is calculated directly from respiration measurements, using the regression method to separate maintenance and growth respiration. In the second approach (CC'), *g* is calculated as the difference between total construction costs and the glucose necessary to provide the C skeletons. In this calculation it was assumed that the organs of herbaceous and woody species have a C concentration of 400 and 450 mg g^{-1}, respectively. For comparative purposes, *g* values are converted from millimoles CO_2 produced per gram of material to gram glucose respired per gram of material formed. Values of *g* were restricted to vegetative organs and are from Hughes (1973), Merino *et al.* (1982), the values listed in Table 5.1 from Amthor (1989), Baker *et al.* (1992), Wullschleger and Norby (1992), Wullschleger *et al.*. (1992), Lehto and Grace (1994), Bunce (1995), Shinano *et al.* (1995), and Wullschleger *et al.* (1995); as well as obtained from D. Garcia (personal communication). Data for construction costs are from the compilation listed in the legend of Fig. 6. Values above the box plots are averages for the category of interest.

independently are scarce. Merino *et al.* (1984) calculated the theoretical *g,* based on chemical composition, for three chaparral species. These values were close (within 7%) to the *g* estimates obtained by the regression method for the same leaves (Merino *et al.,* 1982). More or less similar conclusions could be derived from data by Marcelis and Baan Hofman-Eijer (1995) and Shinano *et al.* (1995).

C. Differences between Organs

Griffin (1994) reviewed the literature on construction costs obtained by calorimetric estimates. He concluded that there is substantial divergence in construction costs between species. However, Poorter (1994), reviewing literature data on construction costs estimates from all available methods, was unable to find systematic differences between functional groups of species and thus concluded the opposite. Since that time, the amount of data has doubled. In this section, we update the review of Poorter (1994). A compilation of these data is given in Fig. 6, with new references listed in the legend.

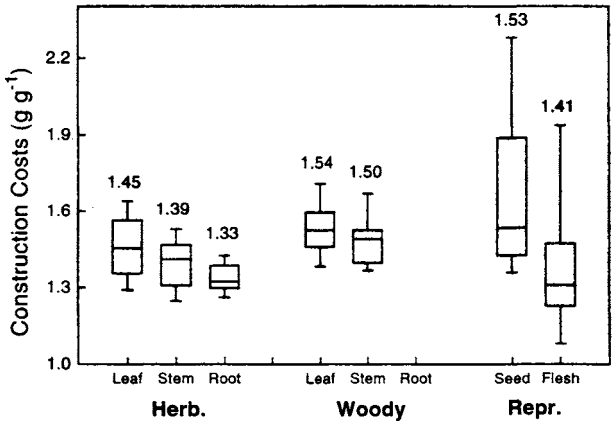


Figure 6 Characterization of the distribution of the construction costs (grams glucose required per gram of biomass formed), by box plots. Data are from the compilation by Poorter (1994) and are supplemented by those of Amthor *et al.* (1994), Sobrado (1994), Walton and Fowke (1995), 76 species from Villar (1992), 27 species (Poorter *et al.,* 1997), 10 species (H. Poorter and J. R. Evans, unpublished), and 60 boreal species (V. I. Pyankov and H. Poorter, unpublished). Furthermore, construction cost values were calculated from Waterman *et al.* (1980), Jordano (1995), and Shinano *et al.* (1995). The total number of observations are as follows: herbs, leaf 157, stem 32, root 32; woody species, leaf 203, stem 26; reproductive organs, seed 47, fruit flesh 294. Values above the box plots are averages for the category of interest.

Most of the research up to now has focused on the construction costs of leaves only. Based on all available data from herbaceous and woody species, we conclude that construction of 1 gram of an average leaf requires 1.50 g glucose (see Fig. 6). There are some data available where stems and roots have been analyzed as well. Generally, stems have somewhat lower construction costs (1.45 g glucose g^{-1}) than leaves. Roots have hardly been investigated, but estimates cluster around 1.33. This could be explained by the difference in chemical composition, as shown in Fig. 1 and Table I. Stems and roots do have lower levels of the expensive compounds lipids and protein, but higher levels of lignin. Concentrations of cheap compounds show differences as well, with organic acids being lower but minerals being higher. Clearly, not just one compound determines the difference. Rather, it is the balance between the various constituents which causes the organs to differ in construction costs. Not much data are present to make inferences on systematic variation in construction costs between organs of woody species. Notwithstanding the high lignin concentrations (Fig. 1A), stems of woody species do not show particularly high construction costs (Fig. 6). This is due to the accompanying large accumulation of TSC.

On average, seeds have similar costs as leaves, although the variation in values is much larger. Especially in seeds with both a high lipid and protein content, construction costs can be over 2 g glucose g^{-1}. Values of the fruit flesh are somewhat lower than those of seeds, although again variation is much larger than in vegetative organs (see Fig. 1).

D. Interspecific Variation

Interspecific variation can be considerable, if reports on individual species are compared. In some cases, values for leaves as low as 1.1 or as high as 2.0 g glucose g^{-1} have been reported. This led Griffin (1994) to the conclusion that there was substantial divergence in construction costs between species. Unfortunately, these reports generally comprise single determinations, so there is no clue as to how consistent these differences are. Furthermore, as a proximate chemical analysis is lacking, there is no clue to the underlying reasons for the low or high values. When construction costs are compared between functional groups of species, differences are small. However, contrary to the previous compilation of Poorter (1994), we detected some systematic interspecific variation. On average, leaves of woody species have 6% higher construction costs than those of herbaceous species (1.54 versus 1.45 g glucose g^{-1}; $P < 0.001$). However, it should be remembered that in this data set all kinds of values are compiled. To what extent are these observations backed up by larger scale comparisons? When we analyze all data on deciduous and evergreen woody species, in reports where both life forms were investigated simultaneously, deciduous species

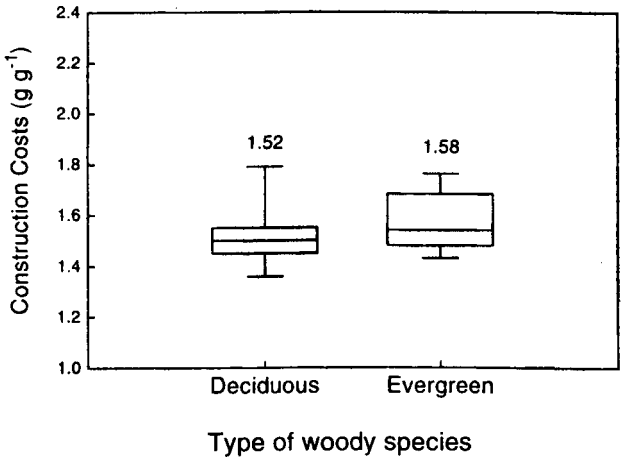


Figure 7 Characterization of the distribution of construction costs (grams glucose required per gram of biomass formed), by box plots, for leaves of deciduous (n = 36) and evergreen (n = 35) woody species. Data are from Merino *et al.* (1984), Merino (1987), Chapin (1989), Villar (1992), and Sobrado (1994). Values above the box plots are averages for the category of interest.

show 4% lower leaf construction costs than evergreens ($P < 0.05$; Fig. 7). In a survey of woody species across Spain, Merino (1987) found that gymnosperms had higher leaf construction costs than deciduous angiosperms (11% difference) and that evergreen species had higher leaf construction costs than deciduous plants (7% difference). Chapin (1989) did not find systematic differences between arctic plant species of different life forms. In all of these cases variation in environmental parameters may have affected the results. However, a similar difference (11%, $P< 0.01$) between six herbaceous and four woody species was found when plants were grown in growth rooms (H. Poorter and J. R. Evans, unpublished). Therefore, we conclude that there is at least some indication of a small inherent difference in construction costs between leaves of herbaceous and (evergreen) woody species.

Within the group of herbaceous angiosperms, Poorter and Bergkotte (1992) did not find a systematic relationship between leaf and/or plant construction costs and the potential growth rate of 24 species. No other group differences have been explored so far.

The reason for construction costs of vegetative organs being relatively constant is the pattern of covariation between the various classes of constituents (Section IV). In arctic plants, Chapin (1989) observed negative correlations between expensive compounds like protein and lignin, or

lignin and tannin. Poorter (1994) concluded that such negative relationships existed in fast- and slow-growing herbaceous species as well, but that quantitatively they could not explain the absence of differences in construction costs. Rather, it was the positive correlation between expensive proteins show 4% lower leaf construction costs than evergreens ($P < 0.05$; Fig. 7). In a survey of woody species growing across Spain, Merino (1987) found and cheap minerals that was the main reason for the lack of systematic variation. As noticed before, interspecific variation in construction costs of reproductive organs is much larger (Fig. 6). This is due to the fact that seeds and fruit flesh may contain large concentrations (up to 800 mg g^{-1}) of storage compounds (Fig. 1), which in some cases are costly (lipids, protein) and in other cases are cheap (TNC).

E. Effect of the Environment

Environmental impact on the construction costs is scarcely investigated, with most of the work again restricted to leaves. As a rule, effects are small (Fig. 8). On average, there was a 3% decrease in construction costs with increases in the ambient CO_2 concentration. The decrease is explained by the accumulation of TNC and, independent of that, a decrease in the concentration of protein (Griffin *et al.,* 1993; Poorter *et al.,* 1997). The effect of light is variable. Williams *et al.* (1989) observed both higher and

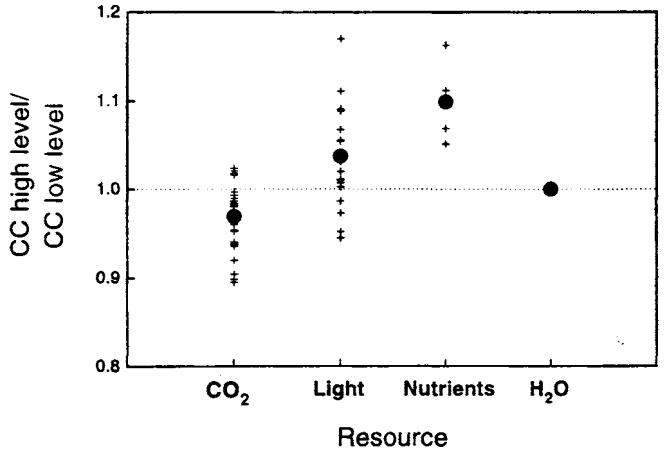


Figure 8 Differences in leaf construction costs (grams glucose required per gram of biomass formed) due to growth of plants under limiting conditions. Data are from Merino (1987), Lafitte and Loomis (1988), Williams *et al.* (1989), Griffin *et al.* (1993), Amthor *et al.* (1994), Griffin (1994), Sims and Pearcy (1994), Shinano *et al.* (1995), Poorter *et al.* (1997); 27 species grown at 350 and 700 µl liter^{-1} CO_2), and H. Poorter and J. R. Evans (unpublished,10 species at light intensities of 200 and 1000 µmol quanta m^{-2} s^{-1}). Small pluses indicate ratios observed for leaves of individual species; closed circles indicate the back-transformed average of the In-transformed individual data. Values above the box plots are averages for the category of interest.

lower construction costs for *Piper* species growing in the field in large versus small gaps. In the controlled experiment outlined above, H. Poorter and J. R. Evans found leaf construction costs to be 4% higher across species for plants grown at a light intensity of 1000 μmol quanta m^{-2} s^{-1} as compared to plants grown at 200 μmol m^{-2} s^{-1}. This scales with the lower concentration of minerals in the leaves of high-light-grown plants.

High levels of N increased construction costs by ~10%. Again, changes in the concentrations of TNC, protein, and minerals will be the main factors, with decreases in the first parameter and increases in the other two. An interesting experiment has been carried out by Peng *et al.* (1993), who grew *Citrus* plants with and without mycorrhiza, at both low and high P. Mycorrhizal roots of high-P plants had lower construction costs than those of high-P plants, which could be ascribed to a lower degree of infection with lipid-rich fungal hyphae. However, it should be noted that also nonmycorrhizal roots at high P had lower construction costs than low-P roots. This is at variance with the increases in leaf costs observed for plants grown at high N availability. Without knowledge of the chemical composition of the *Citrus* roots, it is impossible to assess the reason for this difference in behavior. Controlled experiments with water availability have not been carried out. Merino (1987) reports no differences between plants growing in xeric and in mesic habitats.

VII. Ecological Consequences

Based on the literature available at that time, Poorter (1994) concluded that there was hardly any indication for differences between groups of species. We find an indication that leaves of evergreen shrubs and trees have somewhat higher construction costs than leaves of deciduous species. Moreover, we find higher leaf construction costs for high-light plants, and lower costs for high-CO_2 plants. However, differences are small (well below 10%). Does that mean they are insignificant in relation to growth? Assuming all other parameters are constant, we can assess the effect of a given difference in construction costs on growth with help of Eq. (2). A 10% increase in construction costs translates into a 10% decrease in RGR. In the comparison of fast- and slow-growing species, which may differ in RGR by more than 300% (Grime and Hunt, 1975; Poorter and Remkes, 1990; Gamier, 1992), such a difference is small. However, we cannot exclude the possibility that, in some habitats, such a difference might make for a decisive impact on competition. A similar conclusion may be drawn from the range in construction costs observed for individual species. Given the 27% difference between the 10th and the 90th percentile of the distribution of herbaceous

leaf construction costs (Fig. 6), we conclude that in some specific cases the difference in construction costs could lead to a moderate difference in growth.

The costs assessed in this way are the direct costs a plant has to make to construct an organ with a specific chemical composition. However, there are indirect costs and consequences of that as well. If a plant would invest more in protein, it may gear up the photosynthetic and biosynthetic machinery, and in this way increase its growth rate. Alternatively, it may invest more in expensive compounds like lignin and phenolics, which may decrease the risk of herbivory (Bryant *et al.,* 1983; Bazzaz *et al.,* 1987) and increase resistance against decomposition (Enríquez *et al.,* 1993). The relation between chemical composition and these ecologically very important parameters warrants a separate review. We will refrain from that here and only point out that, as a consequence of a high investment in cell-wall compounds and "defense," the proportion of cytoplasmic compounds in the plant decrease, and consequently its rate of C fixation and nutrient acquisition. It will depend on a plant's environment whether such a decreased C acquisition really will confer a cost.

Apart from resistance against herbivory and decomposition, there is one other species attribute that is strongly related to the observed differences in chemical composition: leaf life span. Leaf life span is an important ecological factor in itself, which may affect the outcome of competition (Berendse and Elberse, 1989). Leaf longevity is high for species with a suite of traits characteristic of species from nutrient-poor environments: a low specific leaf area, a low water content per unit dry weight, and a high investment in cell-wall compounds (Lambers and Poorter, 1992; Reich *et al.,* 1992). Contrary to the mechanistic insights into herbivory and decomposition, we have no clue to the mechanisms that determine leaf (and fine root) longevity. Establishing the relative role of morphology, chemical composition, and the genetic program would be one of the areas where ecophysiology could contribute to ecological insights.

VIII. Summary

In this chapter we analyzed the chemical composition of plants by categorizing compounds into eight distinct classes: lipids, soluble phenolics, protein, lignin, total structural carbohydrates (TSC), total nonstructural carbohydrates (TNC), organic acids, and minerals. First, we assessed the concentrations of these compounds in leaves, stems, and roots of herbaceous and woody species, and seeds and fruit flesh. Concentrations of lipids, organic acids, soluble phenolics, and protein are higher in leaves, whereas TSC, lignin, and TNC are generally higher in stems and roots. Woody

species have lower concentrations of protein, minerals, and organic acids, and higher levels of soluble phenolics.

Concentrations of the different compounds do not vary independently of one another. We investigated the patterns that emerge from two larger scale comparisons across species. Some species have high concentrations of protein, minerals, as well as organic acids (cytoplasmic plus vacuolar compounds), whereas others have relatively high concentrations of lignin and TSC (cell-wall compounds). These patterns coincide with the water content of the plant material, the leaf area: leaf weight ratio (specific leaf area), as well as the potential relative growth rate of these species. We discussed a number of mechanistic interpretations for these results but concluded that there is insufficient insight into the regulation of the chemical composition.

Depending on their biosynthetic pathway, the various classes of compounds require different amounts of glucose to provide for C skeletons, reducing power, and energy. The total amount of glucose required to construct 1 gram of a compound yields the construction costs of that compound. These construction costs vary 3-fold for organic compounds, and are basically nil for the minerals. Consequently, construction costs for 1 gram of biomass will depend on its chemical composition. Although large differences in chemical composition are found among species or between plants grown in different environments, differences in construction costs of biomass are generally small (up to 10%) or nonexistent. The positive correlation between expensive and cheap compounds (proteins and minerals/organic acids) or the negative correlation between various expensive ones (protein and lignin) explains the relatively stable construction costs when groups of functional types of species are compared. However, there are some reports on relatively high or low construction costs for leaves of specific species, which warrant more attention. The same holds in general for stems and roots, which are hardly investigated.

IX. Appendix 1

Short Overview of the Chemical Determinations

This is a short overview of the methods currently used in the Utrecht University laboratory to determine the different fractions of plant compounds (see also Fig. 9). Especially with respect to sugars and phenolic compounds, it is advisable to use freeze-dried material. To avoid too many determinations and to ensure sufficient biomass, we pool (for each organ separately) dried biomass from different individuals, to arrive at two independent samples per species, treatment and harvest. Each of these samples is ground to pass through a 0.08-mm sieve. Total dry weight (DW) of each

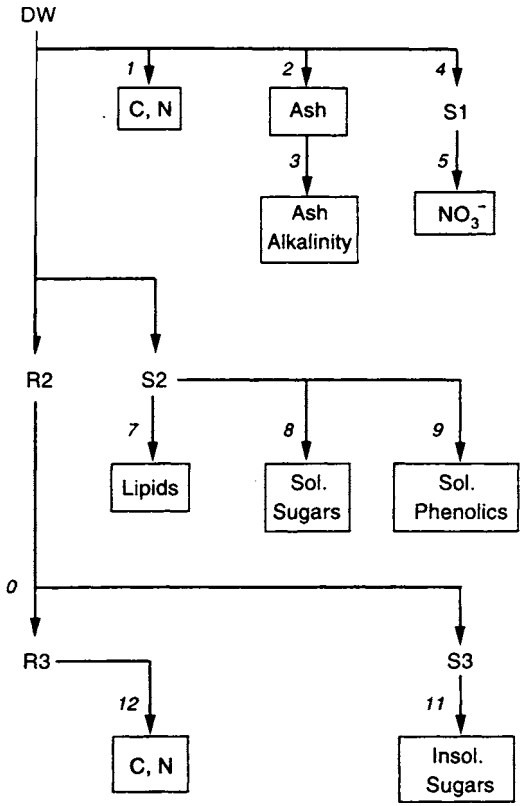


Figure 9 Flowchart of a convenient procedure for determination of the proximate chemical composition. A description is given in Appendix 1.

of the subsamples after redrying should be ~750 mg. This will allow each of the subsequent determinations to be carried out in duplicate. Calculations are discussed in Appendix 2.

Total C and N are determined with an elemental analyzer, which requires 1 mg of material per sample (*1,* numbers refer to Fig. 9). A second fraction, at least 100 mg, is weighed, ashed at 550°C in a muffle furnace, and weighed again *(2).* The ash consists partly of minerals, partly of oxides that are derived from organic acids, and nitrate. These compounds disappear during ashing except for O^{2-} (Dijkshoorn *et al.,* 1968). On cooling the oxides react with CO_2 to form CO_3^{2-}. The total amount of carbonates is determined by quantitatively transferring the ash in an Erlenmeyer flask and determining ash alkalinity by first adding 0.05 *N* HC1 and thereafter titrating back with 0.05 *N* NaOH *(3).* Before being able to calculate total mineral concen-

tration and organic acid concentrations, NO_3^- has to be determined as well. Approximately 25 mg per sample is weighed, and the nitrate is extracted with water at 80°C (*4*). Subsequently, concentrations are determined in the supernatant S1 following Cataldo *et al.* (1975; *5*).

The above determinations are enough to arrive at an estimate of the construction costs. If the aim is to determine the proximate composition of the plant material, then another sample of the ground and redried biomass (±250 mg) is extracted with a mixture of chloroform/methanol according to Bligh and Dyer (1959; *6*). Addition of water produces a chloroform phase and a methanol/water phase. The chloroform is evaporated with N_2 and the residue weighed. This residue largely contains phospholipids and galactolipids, as well as some sterols, and is termed lipids in this chapter (*7*). The water/methanol phase contains the soluble sugars (glucose, sucrose, soluble fructan, etc.), which can be determined with the anthrone method (*8*; Fales, 1951). In the same phase are the soluble phenolics, which can be measured with the Folin-Ciocalteu reagent *(9)*.

The residue R2, mainly cell debris left over after extraction with chloroform/methanol, is boiled for 3 hours at 100°C with 3% HC1. This will break down starch as well as the remainder of the fructans, pectins, and some part of the hemicellulose *(10)*. The sugars released on acid hydrolysis are determined with the anthrone method *(11)*. The residue that is left over after this extraction, R3, consists of cellulose, hemicellulose, cell wall protein, protein that had precipitated during the first extraction, and lignin. After drying and weighing this residue is pulverized, and a sample is taken for a C and N analysis in the elemental analyzer *(12)*.

X. Appendix 2

Calculations

The concentrations of lipid, soluble phenolics, and soluble and insoluble sugars are determined directly either by weighing or with calibration curves. Protein is calculated by subtracting NO_3^- N from total N and multiplying this difference by 6.25. Organic acid concentration (OA) is derived from ash alkalinity (AA), expressed in mEq g^{-1}, and the NO_3^- concentration, also expressed in mEq g^{-1}:

$$\mathrm{OA} = \frac{\mathrm{AA} - \mathrm{Nit}}{62}, \tag{A1}$$

where 62 is the weight of 1 Eq of organic acids. This number 62 is the average value determined by gas chromatography for some grasses grown hydroponically and may be different in cases where malate is not the main organic acid.

The mineral concentration (Min) is calculated as

$$\mathrm{Min} = \mathrm{Ash} - \mathrm{AA} \times 30 + \mathrm{Nit}, \tag{A2}$$

where Ash and Nit are expressed as mg g^{-1} dry weight, and ash alkalinity (AA) expressed in mEq g^{-1} is multiplied by the weight of carbonate per equivalent of charge.

When the concentrations of C, organic N, and minerals (Min; all in mg g^{-1}) are known, the construction costs (in g glucose g^{-1}) can be calculated (Vertregt and Penning de Vries, 1987; modified by Poorter, 1994) as follows:

$$\mathrm{CC} = \left(-1.041 + 5.077 \times \frac{\mathrm{C}}{1000 - \mathrm{Min}}\right) \times \frac{1000 - \mathrm{Min}}{1000} + \left(5.325 \times \frac{N_{org}}{1000}\right). \tag{A3}$$

The available methods to determine lignin are generally rough, use very aggressive chemicals, show interference with other plant compounds, and have to be calibrated with standards (e.g., Morrison, 1972; Morrison *et al.,* 1995). As no method currently exists to obtain pure lignin, these standards are not precise either and generally consist of a gravimetrically determined residue of plant material treated with a series of increasingly aggressive chemicals. Another drawback of this method is that calibrations actually have to be carried out for each species separately, which makes comparative analyses extremely tedious. Therefore, we chose to calculate lignin concentrations in a quicker and easier way, which, though not fully precise either, avoids a number of the above problems. The essence of this calculation is that lignin has an almost 50% higher C concentration than the TSC complex. Starting from the C concentration of R3 *(C_{R3};* see Fig. 9), we can arrive at an estimate of the lignin concentration. Given the total weight of the protein fraction, and the concentration of C in plant protein in general *(C_{prot};* 530 mg g^{-1}) we can calculate the C concentration of the nonprotein fraction of the residue, *C'*:

$$C' = \frac{C_{R3} \times W_{R3} - C_{prot} \times N_{R3} \times 6.25}{W'_{R3}} \tag{A4}$$

where W_{R3} is the weight of residue R3. The weight of the nonprotein fraction, W'_{R3}, is given by

$$W'_{R3} = W_{R3} - N_{R3} \times 6.25. \tag{A5}$$

We assume that this fraction consists of two compounds only: TSC (W_{TSC}) and lignin (W_{Lig}):

$$W'_{R3} = W_{TSC} + W_{Lig}. \tag{A6}$$

The C concentration of this fraction will depend on the relative contribution of TSC (with a C concentration C_{TSC}) and the lignin fraction (with a C concentration C_{Lig}):

$$C' = \frac{W_{TSC}}{W'_{R3}} \times C_{TSC} + \frac{W_{Lig}}{W'_{R3}} \times C_{Lig} \quad \text{(A7)}$$

The TSC complex and lignin both have variable C contents, depending on the relative contribution of their building blocks. However, values of 440 and 640 mg g^{-1} (Fengel and Wegener, 1989) are reasonable approximat- ions to work with. Then, there are two equations [Eqs. (A6) and (A7)] with two unknowns. The lignin concentration in the pellet can be calculated as

$$\frac{W_{Lig}}{W'_{R3}} = \frac{C' - C_{TSC}}{C_{Lig} - C_{TSC}} \quad \text{(A8)}$$

TSC, forms the remainder. Given the concentrations in the pellet, concentrations of lignin and TSC in the total dry weight sample can be readily calculated.

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