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A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity

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SUMMARY

Laboratory experiments have shown a large difference in specific leaf area (SLA, leaf area:leaf mass) between species from nutrient-poor and nutrient-rich habitats, but no systematic difference in the construction costs (the amount of glucose required to construct 1 g biomass). We examined how far these patterns are congruent with those from field-grown plants. An analysis was made of the vegetation in a range of grasslands and heathlands differing in productivity. The SLA of the dominant species in 15 different habitats was determined, as well as chemical composition and construction costs of bulk samples of leaves. SLA in the field was generally lower than in the laboratory, but showed consistency in that the ranking across species remained the same. Species from highly productive habitats had higher SLA than those from sites of low productivity, although individual species sometimes deviated substantially from the general trend. Construction costs were similar for plants from different habitats. This was mainly due to the positive correlation between an expensive class of compounds (proteins) and a cheap one (minerals).

Key words: chemical composition, construction costs, productivity, specific leaf area, comparative approach, vegetation, field-grown plants, laboratory-grown plants.

INTRODUCTION

Plant species show wide variation in their potential relative growth rate (RGR) when grown under near-optimal conditions (Grime & Hunt, 1975; Chapin, 1980; Poorter, 1989). In particular, plant species normally found in nutrient-poor, unproductive habitats have low potential RGR, whereas species generally occurring in nutrient-rich, productive habitats tend to have inherently high RGR. Given the strong correlation between a species' potential RGR and its occurrence in specific habitats, it has been suggested that potential RGR has been the target of selection in these cases (Grime & Hunt, 1975; Chapin, 1980). Alternatively, it may be not RGR so much as one of the components underlying RGR that has been the target of selection (Lambers & Dijkstra, 1987; see also Grime, 1979; Coley *et al.*, 1985). Tilman (1988) suggested that the allocation of biomass is different between species from different habitats, with species in productive environments (where relatively strong competition for light occurs) having a high allocation to leaves and stems, and species from unproductive environments (where there is relatively stronger competition for nutrients) allocating inherently more biomass to roots. In an analysis of the inherent variation in growth characteristics of 24 herbaceous species grown at a non-limiting supply of nutrients, there was indeed some correlation between the potential RGR of a species and the leaf mass fraction (the fraction of biomass allocated to leaves; Poorter & Remkes, 1990). However, this correlation was much weaker than that between the potential RGR and the specific leaf area (SLA, leaf area:leaf mass) of the species. This conclusion was corroborated in a survey of 111 published comparative growth experiments on herbaceous species, where again SLA was the predominant factor explaining variation in RGR

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(Poorter & Van der Werf, 1998). Based partly on these observations, it has been suggested that SLA, or more precisely factors pertaining to a complex of traits related to SLA, could have been the target of selection (Poorter, 1989; Van der Werf *et al.*, 1993; Poorter & Garnier, 1999). Generally, high-SLA species are characterized by high concentrations of nitrogen; high rates of CO_2 and N uptake per unit leaf and root mass, respectively; and a high rate of photosynthesis per unit leaf N (Lambers & Poorter, 1992). These species are geared for a high rate of resource acquisition. Low-SLA species, on the other hand, generally have high values for dry matter content (dry mass : fresh mass); high concentrations of cell walls and secondary compounds; and greater leaf and root longevity (Coley *et al.*, 1985; Choong *et al.*, 1992; Ryser, 1996; Reich, 1998). These species seem to be geared for the conservation of acquired resources (for reviews see Aerts & Chapin, 1999; Poorter & Garnier, 1999).

Most of the above-mentioned analyses on the causes of interspecific variation in growth rate have been carried out in growth rooms or glasshouses, with a relatively high supply of nutrients and water and a relatively low irradiance (Garnier & Freijsen, 1994). Inferences on a possible role of SLA and the suite of related traits as important determinants of plant functioning remain rather speculative if they are based on observations of plants grown under controlled conditions. Therefore, the first question to analyse is how far SLA data from laboratory-grown plants are indicative of SLA values of plants growing in the field. Are the values similar, and does the ranking across species remain the same? The second question relates to the presumed correlation between SLA and habitat productivity. Most of the suppositions regarding a positive correlation between SLA (or potential RGR) and habitat productivity have been based on qualitative impressions (e.g. Grime & Hunt, 1975) or were inferred from semi-quantitative data such as the nitrogen numbers of Ellenberg (Poorter & Remkes, 1990; Fichtner & Schulze, 1992; Van der Werf *et al.*, 1998). It remains to be seen whether there is indeed a positive correlation between SLA and a more quantitative estimate of habitat productivity. Moreover, if any relationship does exist, it is of interest both to assess the form of the relationship (linear, saturating), and to investigate how far individual species may deviate from the general trend. To this end, we determined the SLA of around 70 species from a range of Western European grasslands and from some ericaceous vegetation types. These habitats differ in productivity, due mainly to variation in nutrient availability.

A third concern addressed in this paper regards the construction costs of leaves (the amount of glucose required to construct all of the chemical constituents of 1 g of leaf). Plant species from unproductive habitats tend to accumulate many carbon-based secondary compounds, including lignin and tannins (Coley *et al.*, 1985; Lambers & Poorter, 1992). These compounds have high specific costs of construction, and it has been suggested that leaves of species accumulating these compounds therefore have high construction costs (Miller & Stoner, 1979). However, leaves of these species generally also have low concentrations of proteins, another group of compounds that are costly to produce (Penning de Vries *et al.*, 1974). Thus it may well be that variation in construction costs between species is only small (Chapin, 1989). For species grown with a non-limiting nutrient supply under controlled conditions, no relationship was observed between the potential RGR of a species and the construction costs of the leaves (Poorter & Bergkotte, 1992). Few data are available for leaf construction costs of plant species growing *in situ* in habitats differing in productivity. Therefore the third aim of this paper is to quantify the construction costs of leaves in productive and unproductive habitats. To determine the underlying reasons for possible variation in construction costs, a proximate analysis was carried out of the quantitatively important compounds in the leaf biomass of these vegetation types. For these analyses, all leaves were bulked from within a stand, precluding an assessment of variation between species within a vegetation type.

MATERIALS AND METHODS

Design of the study

Fifteen habitats expected to differ in productivity were selected, all in the central or southern part of the Netherlands. The vegetation in 11 of these habitats is grassland (generally mown once or twice a year). In addition, two ruderal vegetation types in highly disturbed areas and one dry and one wet ericaceous vegetation were chosen (Table 1). The first sampling period was relatively early in the growing season (late April, 1993) and included eight of the habitats. The second sampling period was when the vegetation approaches peak biomass (early July, 1993) and included all 15 habitats. At each time, species were selected that occurred relatively frequently in the habitat or had relatively high biomass. The number of species sampled per habitat varied between one and eight, with an average of five.

For each of the species selected, five individual shoots were randomly chosen, and the youngest fully expanded leaf as well as the oldest green and viable leaf were selected. Hereafter, these leaves will be referred to as 'young' and 'old', respectively. Two categories of leaves were taken to ensure that at least two phases in the life span of the leaves were represented in the analysis. In the case of species

Table 1. *Characterization of the 15 selected sites, topographical location and average estimated above-ground biomass produced per year (EABP ± SD, expressed as percentage of the mean; n = 2)*

Habitat or vegetation type	Location	Coordinates (°E, °N)	Also sampled in first period	EABP (g m^{-2} yr^{-1})
1. Drifting sand dune	Kootwijkerzand (Gld)	5° 47′, 52° 11′	*	90 ± 22%
2. Quaking fen	Westbroekse zodde (Utr)	5° 07′, 52° 10′	*	100 ± 3%
3. Grass heath	Loobosch (Gld)	5° 44′, 52° 10′		130 ± 4%
4. Wet heath	Luttenbergerven (Ovr)	6° 21′, 52° 24′	*	200 ± 29%
5. Dry heath	Wolfhezer heide (Gld)	5° 47′, 52° 00′	*	220 ± 9%
6. Ruderal (trampled)	Uithof (Utr)	5° 10′, 52° 05′	*	280 ± 11%
7. Dry open grassland (south-facing slope)	Berghofweide (Lim)	5° 53′, 50° 50′	*	300 ± 12%
8. Chalk grassland (north-facing slope)	Gerendal (Lim)	5° 52′, 50° 51′		330 ± 10%
9. Poor haymeadow	Luttenbergerven (Ovr)	6° 21′, 52° 24′	*	430 ± 2%
10. Roadside	Rhijnauwen (Utr)	5° 10′, 52° 05′	*	690 ± 9%
11. Ruderal (not trampled)	Westbroek (Utr)	5° 08′, 52° 09′		730 ± 14%
12. Along ditch (never mown)	Lunetten (Utr)	5° 09′, 52° 04′		990 ± 4%
13. Roadside	Amerongse Bovenpolder (Gld)	5° 27′, 52° 00′		1020 ± 22%
14. Fertilized meadow	Uithof (Utr)	5° 10′, 52° 05′		1080 ± n.d.
15. Reed marsh	Westbroekse zodde (Utr)	5° 07′, 52° 09′		1090 ± 33%

with very small leaves, a number of leaves of similar plants were combined. A random sample of five leaves was chosen for those species where old and young leaves could not be distinguished because plants had just developed their leaves.

Twelve of the 24 species grown by Poorter & Remkes (1990) under laboratory conditions (*Cynosurus cristatus*, *Galinsoga parviflora*, *Geum urbanum*, *Hypericum perforatum*, *Origanum vulgare*, *Poa annua*, *Rumex crispus*, *Scrophularia nodosa*, *Briza media*, *Lysimachia vulgaris*, *Phleum pratense* and *Pimpinella saxifraga*) were not among the species harvested in the 15 habitats. To enable a wider comparison between laboratory and field data, SLA values were also collected for the first eight of these species in other habitats, following the sampling scheme described above. Data were included from the literature on the SLA of *Carex flacca* and *Galium aparine*, obtained under exactly the same conditions in the same laboratory by Van der Werf *et al*. (1993) and Den Dubbelden & Verburg (1996), respectively. All SLA values obtained in the laboratory are averages over all viable leaves of the plants.

SLA and productivity

To obtain an impression of the light climate experienced by the leaves, irradiance (μmol quanta m^{-2} s^{-1} in the 400–700 nm range) was determined immediately above each leaf, as well as the irradiance above the vegetation. Measurements were carried out with a LiCor LI 185A (Lincoln, NE, USA). Light measurements were taken between 10:00 and 15:00 with the light sensor in a horizontal position, and no attempt was made to separate the diffuse from the direct site factor. Following light measurement, leaves were collected, wetted and stored in a cool box. At the end of the day leaves were placed between wet tissues and stored overnight in a fridge, after which they were further processed. By storing them wet, leaves could reach equilibrium with free water, correcting for possible differences between habitats in short-term water availability (Eliáš, 1985). The next day petioles and leaf sheaths were separated from the leaf blades and discarded. In the case of very narrow or cylindrical leaves (*Corynephorus canescens*, *Deschampsia flexuosa*, *Festuca ovina*, *Juncus subnodulosus*), the leaf width in the middle part of the leaf was determined, as well as the leaf length. Leaf blade area was then calculated as half the total intercepting area, following Chen & Black (1992). For *Calluna vulgaris* and *Erica tetralix*, length and width of the minute leaves were measured under a microscope, and the area calculated by multiplying length × width with a correction factor that takes into account the form of the leaves. For all other species, leaf area was determined with a DIAS image analysis system (Delta-T, Cambridge, UK; small leaves) or a LiCor (larger leaves). After determination of the area, leaves were dried for at least 48 h at 70°C and dry mass was determined.

The above-ground standing crop was determined in duplicate in the second period by harvesting all aerial biomass (except litter and the moss layer) of a 0.5 × 0.5 m area. As most of these sites have negligible aerial living biomass during the winter season, and were not mown before our measurements, the near-maximal standing crop is a somewhat crude estimate of the above-ground productivity in these habitats. This is denoted as

estimated above-ground biomass produced (EABP). Total net productivity will be higher, both because the root fraction was not considered and because leaf turnover during the growing season was not quantified. Nevertheless, the approach followed should give a fair estimate of the differences in productivity between sites (cf. Aerts & Berendse, 1989). There were, however, three clear exceptions. Firstly, in the case of the *Lolium perenne* meadow, the vegetation was frequently and heavily grazed. Fliervoet (1987) and Meijer (1984) report productivity values of 700 and 1450 g m^{-2} yr^{-1} for non-grazed grasslands of this type. In this study it was assumed that EABP values on the site would have been the average of these two reported values. Any other number over 700 g m^{-2} yr^{-1} does not significantly affect the conclusions drawn in this paper. Secondly, standing crop in the dry and wet heathland systems is approximately constant throughout the year (Aerts & Berendse, 1989). In these cases, the older fraction of the stems and leaves of *Erica tetralix* and *Caluna vulgaris*, produced, it is most likely, in the years before, were separated from the fraction produced in the current year. To arrive at an EABP value, the ericaceous biomass produced in the current year was added to the total biomass of the other plant species harvested in the clipped area.

From each of these bulk samples of vegetation, two independent samples of leaves were randomly selected to obtain sufficient leaf material for the chemical analyses. Thus, whereas the SLA data were collected for each of the species investigated, the chemical analyses are representative for the leaf fraction of the entire vegetation. Dry mass was determined after drying for at least 48 h at 70°C.

Chemical analyses

Each leaf sample of the collected bulk vegetation was ground to pass through a 0.08 mm sieve, and redried. An exact description of the procedures followed for the chemical analyses and the subsequent calculations is given in Poorter & Villar (1997). Carbon and total nitrogen content were measured with an elemental analyser, and ash content was determined by combustion of plant material in a muffle furnace. Ash contains not only minerals, but also oxides of organic acid and nitrate formed during combustion. Therefore we determined ash alkalinity by titration and, in a separate sample, the nitrate content by a colorimetric assay with salicilic acid. Lipids were determined gravimetrically in the chloroform fraction of a chloroform–methanol–water extract. Soluble phenolics were determined in the methanol–water phase with the Folin–Ciocalteu reagent. Soluble sugars were determined in the same extract; insoluble sugars after boiling with 3% HCl. Sugars were determined with anthrone. The residue left over after the chloroform–methanol extraction and the 3% HCl treatment was considered to consist of crude cell walls. From the C and N content of this residue the concentrations of total structural carbohydrates (TSC) and lignin were derived. The only adjustment made to the scheme given by Poorter & Villar (1997) was for minerals, possibly silica, which appeared to be present in the crude cell wall residue. To correct for this, the ash content in the crude cell wall fraction was determined, and the C and N concentration in this fraction adjusted accordingly. All determinations were carried out in duplicate on each of the independent bulk samples.

Calculations and statistics

Protein concentration was calculated by subtracting nitrate-N from total N, and multiplying by 6.25. Organic acid concentration was estimated by subtracting nitrate content (in meq g^{-1}) from ash alkalinity, and multiplying by an average molecular weight of 62.5. Mineral concentration was calculated by multiplying ash alkalinity (in meq g^{-1}) by 30 g eq^{-1} (mass of carbonate), subtracting this value from total ash, and adding the weight of nitrate. Lignin concentration was estimated from the C and N concentration in the crude cell wall residue, assuming a C concentration in lignin of 640 mg g^{-1}, and a C concentration in the (hemi)cellulose complex of 444 mg g^{-1} (Poorter & Villar, 1997). Construction costs (K) were calculated with the following formula:

$$K = (-1.041 + 5.077C_{om})(1-M) + (5.325N_{org}) \quad \text{(Eqn 1)}$$

(Poorter, 1994), where K is the construction cost (g glucose g^{-1} d. wt), C_{om} the C content of the organic matter (g g^{-1}), and M and N_{org} the mineral and organic N concentration of the total dry mass (g g^{-1}), respectively. This is a slightly modified approach to that of Vertregt & Penning de Vries (1987), which assumes that NO_3^- is the N source for the plant. The cost of protein is lower if plants utilize ammonium. As the extent to which the study species take up nitrate and ammonium was not known, these construction costs should be considered to be maximum values.

Statistical tests were carried out using SPSS. Area per leaf was ln-transformed before testing, because proportional differences were considered to be more important than absolute differences. The contribution of habitats and species within habitats to the average SLA per individual was analysed using a nested ANOVA. Graphical analyses showed that a number of relationships were non-linear. Therefore, the sum of squares due to habitats was further analysed with orthogonal polynomials, ranking habitats based on the estimated productivity of each site. Where the quadratic term was found to be significant, a saturating function was fitted to the observed data, of the form $y = C_1 + C_{max}[x/(x+C_2)]$,

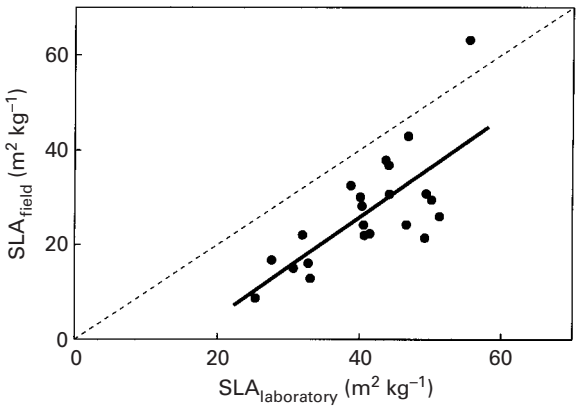

Fig. 1. Relationship between SLA of whole plants grown in the laboratory, and the average SLA of the oldest viable leaf and the youngest fully expanded leaf of the same species grown in the field. Data are for the second sampling period. The broken line is the 1:1 relationship. Total number of species is 22, $r^2 = 0.53$, $P<0.001$. Average SE is 4.8%, with five independent plants measured in the field. Laboratory data for these species are from Poorter & Remkes, 1990 (20 species); Van der Werf *et al.*, 1993 (*Carex flacca*); Den Dubbelden & Verburg, 1996 (*Galium aparine*).

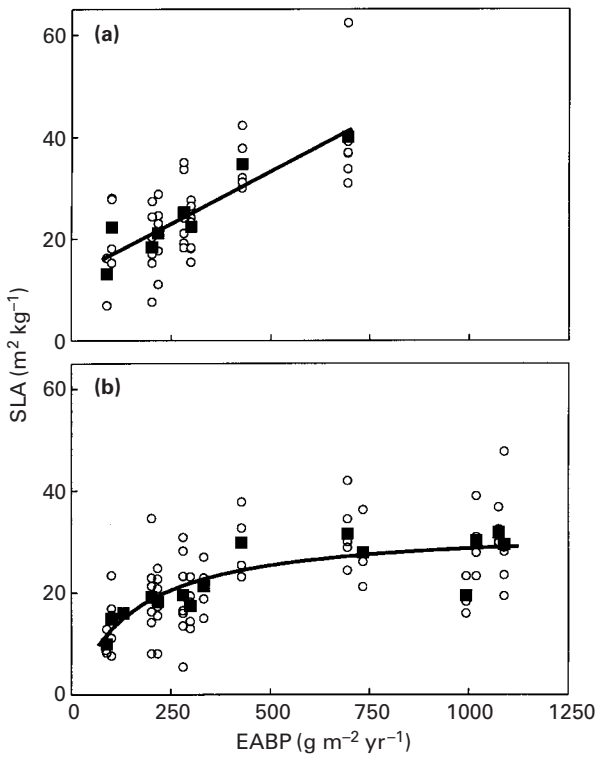

Fig. 2. The SLA values of the dominant species on a site (open circles) and the average values across species within a habitat (closed squares), plotted against the estimated annual above-ground biomass produced (EABP) in a range of habitats. Vegetation types in these habitats consisted of a range of grassland, ruderal and ericaceous stands, measured in (a) late April (eight habitats) and (b) early July (15 habitats). The lines are the linear regressions through the average values per habitat (a: $r^2 = 0.83$, $P < 0.001$; b: $r^2 = 0.92$, $P < 0.001$).

where y is the dependent variable, x the estimated productivity and C_1, C_2 and C_{max} are constants. These constants were estimated with the procedure NLR in SPSS. Where the quadratic term was not significant, data were fitted with a linear regression. Correlations between productivity, SLA, size of leaves and relative irradiance were analysed using path analysis, using standardized coefficients.

RESULTS

SLA and productivity

Generally, recently matured leaves had slightly lower SLA than older leaves (on average 4%); this difference was statistically non-significant. To assess the relationship between the mean SLA of species in the field and in the laboratory, the SLA values were averaged of the youngest fully grown leaf and the oldest still-viable leaf for each individual. These average values were plotted against average SLA data over all viable leaves of plants of the same species, obtained by Poorter & Remkes (1990), Van der Werf *et al.* (1993) and Den Dubbelden &

Table 2. *ANOVA with average SLA per individual measured during periods 1 (April) and 2 (July) as the dependent variables; habitat, and species nested within habitat, as independent variables*

	Period 1			Period 2		
Factor	SS	df	*P*	SS	df	*P*
Habitat	14100	7	***	15200	14	***
linear	11900	1	***	9100	1	***
quadratic	0	1	ns	2200	1	**
rest	2200	5	ns	3900	12	ns
Error (species within habitats)	9700	39		12400	59	
Species within habitats	9700	39	***	12400	59	***
Error (within cells)	1200	183		2500	293	

The sum of squares explained by habitat is further analysed with orthogonal polynomials, ranking habitats on the basis of the estimated above-ground biomass produced at each site. n.s., not significant; **, $P<0.01$; ***, $P<0.001$.

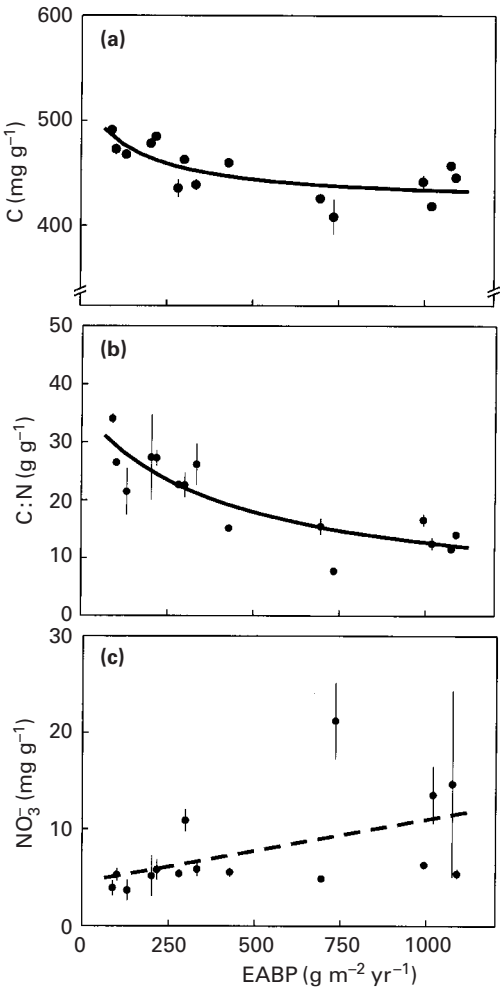

Fig. 3. (a) Carbon concentration, (b) C:N ratio and (c) nitrate concentration of leaves plotted against estimated annual above-ground biomass produced in a habitat (EABP). Data points are the mean values (±SE) from two independent samples of bulked material of all green leaves from all species in that vegetation type. Broken lines indicate a non-significant relationship, continuous straight lines a significant linear regression, and continuous curved lines are saturating curves fitted when the regression analysis showed a significant quadratic component.

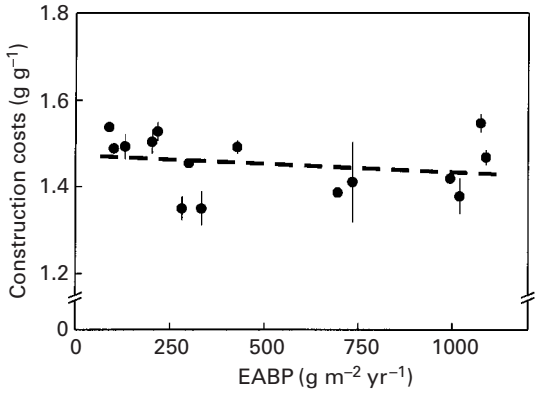

Fig. 4. Construction costs of leaves of different vegetation types plotted against the estimated annual above-ground biomass produced per year in a habitat (EABP). For more information see the legend to Fig. 3.

Verburg (1996) for plants grown in climate chambers at a daily quantum input of 16 mol m^{-2} d^{-1} (Fig. 1). The relation between the SLAs of field-grown and laboratory-grown plants is positive ($P<0.001$), but field-grown plants generally have lower SLA values (39% on average).

Subsequently, an analysis was made of a possible relationship between the estimated productivity of a site and the SLA of the dominant species. Overall, EABP values ranged from less than 100 g m^{-2} yr^{-1} for the vegetation of drifting sand dunes, to more than 1000 g m^{-2} yr^{-1} for a stand of *Phragmites australis* (Table 1). Average SLA values per individual were analysed using ANOVA, with habitat as main factor and species nested within habitats. The main effect habitat explained 50–60% of the total variation in SLA (Table 2). SLA was positively and significantly correlated with the estimated yearly above-ground biomass produced at these sites in both harvesting periods (Fig. 2; see also the first-order polynomial in Table 2). Beyond an EABP value of 500 g m^{-2} yr^{-1}, for which we only have data for the second period, the relationship saturated (Fig. 2b). Variation between species within habitats was considerable, explaining 35–40% of the total variation in SLA (Table 2). This can also be seen from the wide scatter of data points around the mean in Fig. 2.

Construction costs

The C concentration of bulked samples of leaves within a stand correlated negatively with the EABP values of those sites (Fig. 3a, $P<0.01$), data ranging from 410–490 mg g^{-1}. Total N correlated positively with EABP ($P<0.01$, data not shown), and, consequently, the C:N ratio was much higher for sites of low productivity (Fig. 3b, $P<0.001$). Part of the total N (4–12%) was present in the form of nitrate. Although the highest concentrations of nitrate (up to 20 mg g^{-1}) were observed in some of the highly productive sites, no general relationship with EABP was found (Fig. 3c, ns). Leaf construction costs, calculated from the C and organic N values, did show a slightly negative, but non-significant relationship with EABP (Fig. 4, ns). The average value across all habitats was 1.45 g glucose per g leaf dry mass.

Within the group of compounds with low construction costs, highly productive vegetation had markedly higher concentrations of minerals (Fig. 5a, $P<0.01$) and organic acids (Fig. 5b, $P<0.05$). Negative correlations were found for the total non-structural carbohydrates (soluble plus insoluble sugars, Fig. 5c, $P<0.01$) and the amount of total structural carbohydrates (Fig. 5d, $P<0.05$). Within

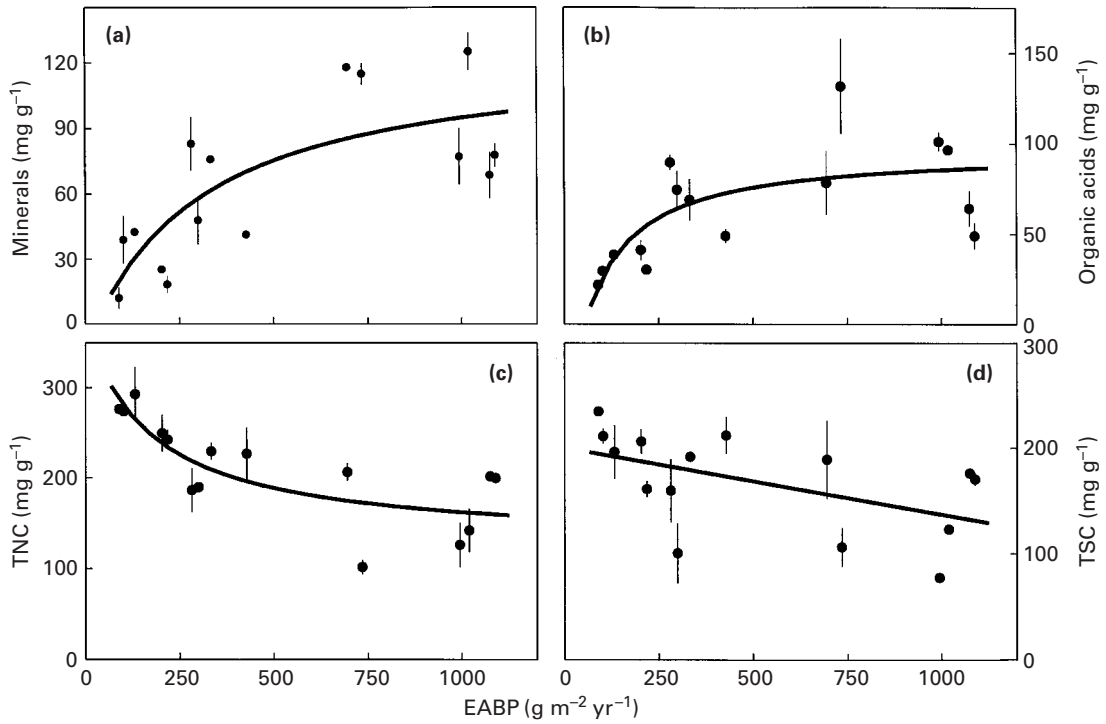

Fig. 5. (a) Mineral concentration, (b) organic acid concentration, (c) concentration of total non-structural carbohydrates (TNC) (starch, fructan, soluble sugars), and (d) concentration of total structural carbohydrates (TSC) (cellulose plus hemicellulose) of leaves plotted against the estimated annual above-ground biomass produced in a habitat (EABP). For more information see the legend to Fig. 3.

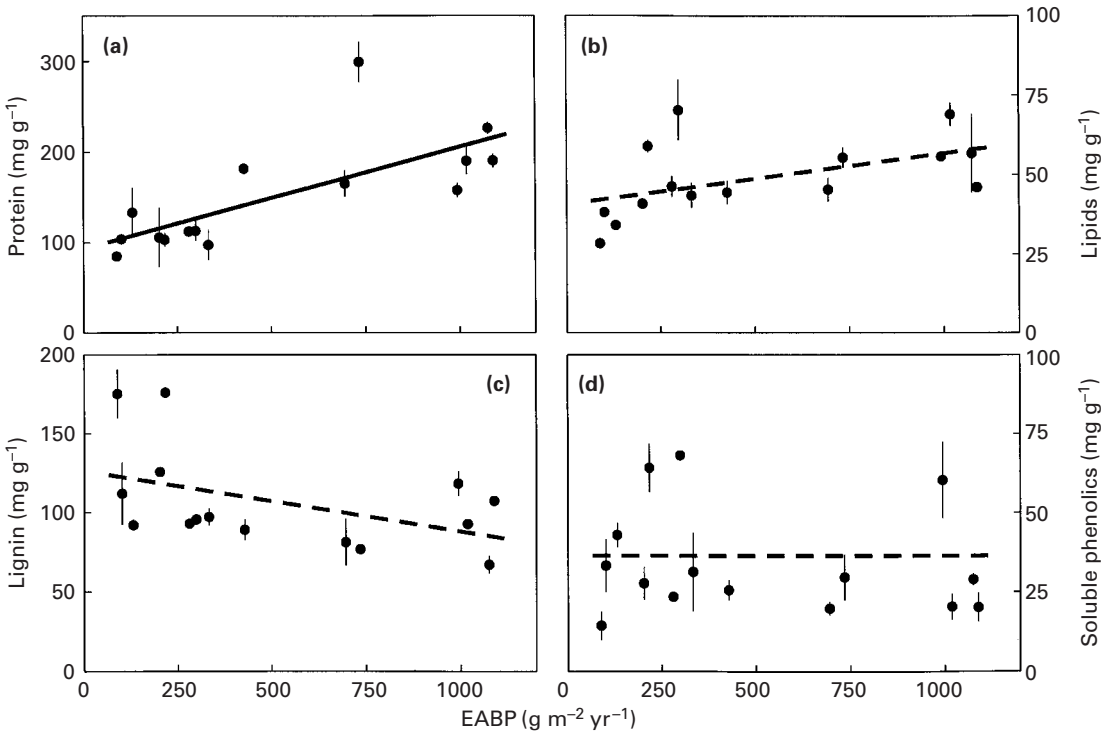

Fig. 6. (a) Protein concentration, (b) lipid concentration, (c) lignin concentration, and (d) concentration of soluble phenolics of leaves plotted against the estimated annual above-ground biomass produced in that habitat (EABP). For more information see the legend to Fig. 3.

the group of expensive compounds, the concentration of protein (Fig. 6a, $P<0.01$) was significantly higher in the highly productive vegetation types, the concentration of lipids was somewhat higher (Fig. 6b, $0.005 < P < 0.10$) whereas lignin concentrations were somewhat lower (Fig. 6c, $0.05 < P < 0.10$). No clear trend was found for the group of soluble phenolics (Fig. 6d, ns).

DISCUSSION

SLA and productivity

There was a positive relationship between the SLA of field-grown and laboratory-grown plants (Fig. 1), with values for field-grown plants being generally lower. Daily quantum input is an environmental parameter that strongly affects SLA (Chabot *et al.*, 1979). As daily quantum input in growth rooms is generally lower than in the field (Garnier & Freijsen, 1994), the SLA of field plants is indeed expected to be lower. The only exception was for *Galinsoga parviflora* (highest value in Fig. 1), which was collected close to a building where sunlight was blocked for part of the day. Apart from the difference in daily light climate, plants outside will also experience more turbulent air movements than those grown under controlled conditions, another factor that may decrease SLA (Woodward, 1983). More important than the absolute SLA values is the relative ranking between field- and laboratory-grown plants, which remains similar. Clearly, genotype × environment interactions were not large; therefore it is concluded that data from comparative laboratory experiments can be used to infer valid conclusions about SLA ranking in the field. Such conclusions are in line with findings in Mediterranean grasslands (Garnier *et al.*, 1997).

The average SLA of the dominant species in the vegetation was positively correlated with the estimated above-ground productivity of that vegetation (Fig. 2), at least up to an EABP value of around 500 g m^{-2} yr^{-1}, after which the relationship saturated. A positive relationship between SLA and EABP was also found by Fliervoet (1987) in an analysis of the vegetation structure in Western European grasslands. Grubb (1977) reports that with increasing altitude, tropical mountain forests decrease in both SLA and productivity. Contrasting evidence comes from the observation that mature forest stands of evergreen conifers and deciduous hardwood species do not differ in productivity, despite strong differences in SLA (Reich, 1998). Reich (1998) explains this by showing that the evergreen species with the low SLA values allocate a relatively large fraction of their total biomass to foliage. This compensates for the lower biomass gain per g foliage in evergreens, to the extent that productivity per unit ground area is similar to that of the deciduous species with the high SLA values. A similar relationship has not been found in grasslands such as those investigated in this study. On the contrary, Fliervoet (1987) reports that in grasslands with a high EABP, a relatively larger fraction of the above-ground biomass is allocated to stems than in less productive grasslands, and a smaller fraction to leaves.

Is the observed relationship between productivity and SLA caused directly by the commonly suggested compromise between resource capture and conservation? And why is the relationship a saturating one? These are difficult questions to answer. The relationship between average SLA and productivity can be modulated by various factors. Leaves of plant species characteristic of productive environments are generally larger (Grime, 1979; Fliervoet, 1987), and therefore may require relatively more support tissue (Givnish, 1986). As support tissue generally consists of dense, lignified material, it could be envisaged that this has a negative effect on SLA (Grubb, 1998), explaining the saturation above an EABP of 500 g m^{-2} yr^{-1}. On the other hand, there might also be a trend towards higher SLA values in productive environments because of the light climate they experience. If lower leaves developed in a period in which they were more-or-less at the top of the canopy, relatively small differences in SLA between newly matured and old leaves can be expected, caused by seasonal differences in light climate during leaf development. However, specific leaf area can partly adjust to changes in light climate that take place after full development (Pons & Pearcy, 1994). The larger the canopy, the less will be the irradiance experienced by the lower leaves. Therefore, one may expect that in highly productive stands (where a large standing crop develops over time), the difference in SLA between old and new leaves will be greater than in plants growing in open vegetation (cf. Niinemets & Kull, 1994).

To correct for the indirect effects that habitat productivity may have on the SLA of the resident species via leaf size, and for the relative irradiance experienced by each leaf, a path analysis was carried out with these four variables as factors of interest (Fig. 7). As this analysis assumes linear relationships, EABP values were ln-transformed. The analysis confirms the observations of Grime (1979) and Fliervoet (1987) that leaf size is larger for plant species from nutrient-rich sites. No negative effect of leaf size on SLA could be detected, in contrast to the

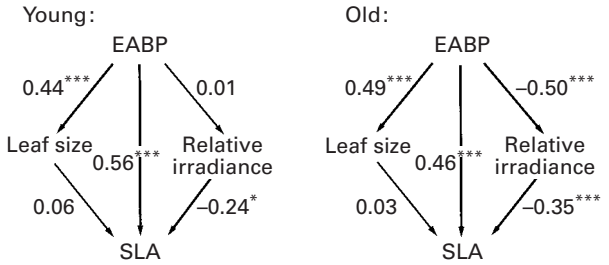

Fig. 7. Path analysis of the SLA data obtained in the second period (early July). Values indicate how much variable *B* changes (expressed in units of SD) when variable *A* is increased by 1 unit of SD. Data were analysed separately for young and old leaves. 'Relative irradiance' is the irradiance at the top of the leaf relative to that above the vegetation. Abbreviations: EABP, estimated annual above-ground biomass produced. Significance levels: *, $P < 0.05$; ***, $P < 0.001$.

observations of Grubb (1998). Does this imply that larger leaves do not require relatively more support tissue than small leaves, or that the support tissue itself is not very dense? Most of the support tissue in leaves is around the mid-vein, and if this is dense material, it should have a negative but local effect on SLA. Therefore, for a range of dicotyledonous species we compared SLA around the mid-veins with that of the leaf lamina, where veins are expected to be less dominant. In line with the result of Shipley & Meziane (1998), SLA was found to be lower around the mid-vein (on average 18%) than in the rest of the leaf (data not shown). However, as the area around the mid-vein is only a small fraction of the total leaf area, the effect on the SLA of whole leaves is small. This may explain why leaf size alone does not have a dramatic impact on SLA.

To correct for differences in light climate due to position in the canopy, measurements were made of the irradiance above the leaf relative to that above the canopy. For young leaves, generally at the top of the canopy, no significant effect of EABP on relative irradiance was found (Fig. 7). Old leaves, however, were more heavily shaded in productive vegetation types. In both cases there was a negative effect of relative irradiance on the SLA of the leaves. The total effect of productivity on SLA via the light climate is found by multiplying the two effects. For young leaves this effect was negligible; for old leaves the shading effect, expressed in units of standard deviation, increased SLA by 0.17 units of standard deviation. Thus in highly productive vegetation types there may be a small effect of the light environment towards an increase in SLA.

A third factor that may influence SLA is the fact that plants from these sites of low productivity are generally nutrient-limited. Within a given species, nutrient limitation may cause SLA to decrease (e.g. Fichtner & Schulze, 1992; McDonald *et al*., 1992), although this response is not always observed (e.g. Sims *et al*., 1998). Nutrient limitation often induces accumulation of total non-structural carbohydrates (TNC) (Poorter & Villar, 1997). Could the lower SLA of plants from environments of low productivity be explained by higher TNC content, analogous to the decrease in SLA for plants grown in elevated CO_2? Considering the leaf fraction of the vegetation as a whole, there was indeed a higher concentration of TNC in the vegetation on low productivity sites (Fig. 5c). We cannot correct for this effect via path analysis as we do not have TNC data for each individual leaf. Assuming that TNC concentration of the plant species for which SLA was measured was the same as for the vegetation as a whole (data in Fig. 5c), we calculated that at most 20% of the difference in SLA between rich and poor sites could be explained by TNC differences. This is an upper limit, as the lower concentration of protein in plants from nutrient-poor sites must also be taken into account. Therefore, it is concluded that this factor cannot be of overriding importance either.

None of the factors discussed above strongly affected the relationship between SLA and productivity. Therefore it is concluded that there is a direct relationship between the SLA of a species and the productivity of the habitats in which these species are generally found. This field survey confirms earlier observations in the growth room, that there are inherent differences in SLA between species from sites differing in productivity. However, two questions remain unanswered. Firstly, we have not been able to explain why the relationship between SLA and productivity saturates above an EABP value of 500 g m^{-2} yr^{-1}. Possibly the differences are strongly related to variation in leaf life span. All of the species in the more productive environments drop most to all of their leaves at the end of the growing season. As there is no strong differentiation in life span between these species (Berendse *et al*., 1998), differences in investment in defence compounds are possibly also small. A second aspect of the relationship between SLA and productivity is that species' variability in SLA within a given site is large, explaining 35–40% of the total variation. This is in line with conclusions from a field survey of Eliáš (1985) and a growth analysis of Van Andel & Biere (1989) under controlled conditions on a range of co-occurring species. Clearly, more factors than SLA are involved in shaping the performance of plants in various habitats. Does this imply that other factors within the suite of traits related to SLA compensate for the fact that one species has a lower SLA than another? If so, it would be of interest to know the trade-offs involved. Alternatively, there may be factors unrelated to growth rate and SLA that explain the success of species at a given site. The suggestion of Westoby (1998) that plant height and seed mass should be included as simple and biologically independent factors to characterize the strategy of a species offers a promising avenue to further our understanding on this point.

Construction costs

Construction costs of the leaves did not vary systematically with the productivity of the habitat (Fig. 4). Thus we can reject the early hypothesis of Miller & Stoner (1979), who expected evergreens from environments of low productivity to have higher construction costs than more productive deciduous species. The only field data relating construction costs of leaves to soil fertility are those of Merino (1987), who made a qualitative assessment and did not find any effect. Controlled experiments measuring the effect of nutrient availability on leaf construction costs within a given species showed small but significant increases with increased N

availability (Shinano *et al.*, 1995; Griffin *et al.*, 1996). When plant species characteristic of low- and high-productivity habitats were grown under controlled conditions at high levels of nutrient supply, no systematic variation in leaf and whole-plant construction costs was found between fast- and slow-growing species (Poorter & Bergkotte, 1992). Chapin (1989) arrived at the same conclusion for various tundra species, all growing in the same habitat. In a review of the literature, Poorter & Villar (1997) concluded that variation in construction costs of leaves is small (within 10%), regardless of the environmental factors assessed.

What causes the construction costs to be constant? To obtain insight into the causes of variation in construction costs, the chemical composition of the biomass has to be known. Variation in leaf chemical composition across habitats is considerable. Leaves sampled in environments of low productivity have high concentrations of C, high C:N ratios, and high concentrations of total non-structural carbohydrates as well as structural carbohydrates (Figs 3a,b, 5c,d). They also have somewhat higher concentrations of lignin (Fig. 6c). This is in line with the carbon–nutrient balance theory (Bryant *et al.*, 1983). Leaves from highly productive sites, on the other hand, have high concentrations of minerals, organic acids, protein and lipids (Figs 5a,b, 6a,b). It is the net balance of all these changes that determines the variation in construction costs. Chapin (1989) concluded that the constancy in construction costs across species was due to a negative correlation between one expensive compound (e.g. lignin) and another (protein). Poorter & Bergkotte (1992), on the other hand, found that the constancy was mainly due to the positive correlation across species between an expensive class of compounds (proteins) and a cheap one (minerals). In both cases the conclusion was drawn from correlations between compounds. However, variation in construction costs depends not only on the direction of changes in compounds, but also on the cost of each of the compounds and the absolute changes across species or habitats.

A quantitative analysis of the changes in chemical composition requires knowledge of the concentrations of all classes of compounds. However, adding up concentrations of the eight classes of constituents, we were not able to account for 100% of the leaf mass, but only for $85 \pm 5\%$. This is in line with previous analyses (87 and 83%, respectively, by Poorter & Bergkotte, 1992 and Poorter *et al.*, 1997), but lower than the 95% reported by Chapin (1989). For the present calculation it was assumed that the missing part could be proportionally distributed over all fractions determined. To determine the reason for the constancy in construction costs, we calculated the differences in chemical composition between vegetation from a typical site of low productivity and a typical site of high productivity.

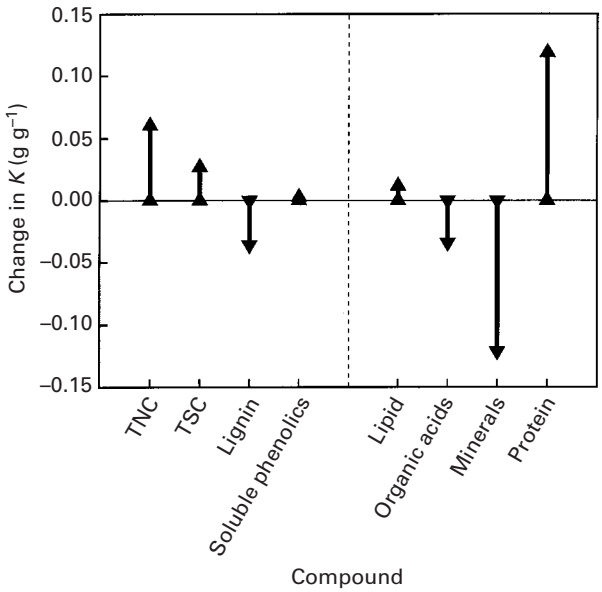

Fig. 8. The effect of differences in the various classes of compounds on the change in construction costs (K). Arrows indicate the value by which construction costs increase or decrease if the concentration of each of compound in the leaf biomass of a typical site of low productivity is altered to the concentration of biomass at the typically highly productive site. The four classes of compounds on the left have a higher concentration in sites of low productivity, whereas the four on the right are higher in highly productive sites. Abbreviations: TNC, total non-structural carbohydrates; TSC, total structural carbohydrates.

These values were based on the lowest and highest values of the regression lines in Figs 5 and 6. Let us assume that compound A was present in a higher concentration in the highly productive site. Knowing the difference in concentration (say 50 mg g^{-1}), we then analysed how construction costs of the leaves at the site of low productivity would change if we replaced 50 mg of the total biomass with 50 mg of compound A. That number gives the direction and the absolute change in construction costs due to a difference in that component. A full explanation of the method followed is given in Appendix 2. Results are shown in Fig. 8, and indicate that leaf biomass at highly productive sites tends to have higher construction costs, because of higher concentrations of the expensive compounds protein and lipid, as well as lower concentrations of the cheap compounds TSC and TNC (upward arrows in Fig. 8). On the other hand, these increases are compensated because leaves in the highly productive habitats accumulate less of the expensive lignin, and relatively more of the cheap classes of organic acids and minerals. Quantitatively, the two most important effects are due to the correlated increases in proteins, with high costs, and cheap minerals, with low costs. These two balance each other and are the major reason that construction costs are constant. Changes in other compounds do play a role as well, but are quan-

titatively less important. Thus it is concluded that these data are in line with the results for fast- and slow-growing species grown at optimum conditions (Poorter & Bergkotte, 1992).

CONCLUSIONS

For the habitats considered in this survey, there is a positive relationship between the SLA of the most common species in a vegetation type and the productivity of the vegetation as a whole. However, within each habitat there is considerable variation in SLA between species. No systematic trend was observed between leaf construction costs and habitat productivity. This was due mainly to the positive correlation in the concentration of an expensive class of compounds (proteins) and a cheap one (minerals).

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Appendix 1. *List of species sampled in the different habitats, and the average SLA of the youngest fully grown and the oldest still-viable leaf for plants sampled in period 1 (April 1993) and period 2 (July 1993)*

Habitat or vegetation type	Species sampled	SLA ($m^2\ kg^{-1}$) Period 1	Period 2
1. Drifting sand dune	*Ammophila arenaria*	6.9	8.2
	Corynephorus canescens	16.2	12.9
	Festuca ovina	16.2	8.7
2. Quaking fen	*Calamagrostis canescens*	28.0	–
	Carex lasiocarpa	18.0	11.1
	Carex nigra	15.2	16.9
	Juncus subnodulosus	–	7.6
	Menyanthes trifoliata	27.8	23.4
	Potentilla palustris	22.4	15.6
3. Grass heath	*Deschampsia flexuosa*	–	16.0
4. Wet heath	*Carex panicea*	20.3	22.9
	Cirsium dissectum	17.0	16.3
	Erica tetralix	7.5	8.0
	Molinia caerulea	24.4	21.3
	Salix repens	15.2	14.2
	Succisa pratensis	15.2	16.3
	Viola palustris	27.4	34.6
5. Dry heath	*Carex pilulifera*	24.6	20.8
	Calluna vulgaris	11.0	8.0
	Deschampsia flexuosa	21.4	17.3
	Galium saxatile	28.8	–
	Genista anglica	17.6	15.5
	Molinia caerulea	23.0	22.7
	Rumex acetosella	–	24.8

Appendix 1. (*cont.*)

Habitat or vegetation type	Species sampled	SLA (m^2 kg^{-1}) Period 1	Period 2
6. Ruderal and trampled	*Cirsium arvense*	19.2	5.4
	Plantago lanceolata	24.1	13.5
	Plantago major	21.1	16.0
	Potentilla anserina	18.2	30.9
	Trifolium repens	33.6	28.2
	Unidentified grass	35.0	23.2
7. Dry open grassland (south-facing slope)	*Anthoxanthum odoratum*	27.6	–
	Centaurea jacea	25.7	16.5
	Festuca rubra	15.4	13.0
	Leucanthemum vulgare	18.1	14.0
	Plantago lanceolata	24.2	19.4
	Rhinanthus minor	26.5	–
	Trifolium pratense	23.3	23.1
	Unidentified Composite	18.3	18.2
8. Chalk grassland (north-facing slope)	*Brachypodium pinnatum*	–	21.9
	Carex flacca	–	15.0
	Centaurea jacea	–	18.8
	Dactylorrhiza maculata	–	22.0
	Leontodon hispidus	–	27.0
	Ononis repens	–	22.9
	Plantago lanceolata	–	21.4
9. Poor hay meadow	*Agrostis tenuis*	–	25.4
	Alopecurus pratensis	30.0	–
	Anthoxanthum odoratum	32.0	30.0
	Holcus lanatus	31.1	37.8
	Lolium perenne	42.2	–
	Leontodon autumnalis	–	32.7
	Ranunculus repens	34.7	23.1
	Taraxacum officinale	37.8	–
10. Roadside	*Aegopodium podagraria*	39.1	30.0
	Anthriscus sylvestris	30.9	30.0
	Arrhenaterum elatius	36.8	34.5
	Dactylis glomerata	36.9	28.9
	Galium aparine	62.3	42.0
	Urtica dioica	33.7	24.4
11. Ruderal (not trampled)	*Brassica napus*	–	26.1
	Chenopodium album	–	21.2
	Polygonum persicaria	–	36.3
12. Along ditch (never mown)	*Cirsium arvense*	–	16.0
	Epilobium hirsutum	–	19.7
	Phragmites australis	–	18.3
	Urtica dioica	–	23.3
13. Roadside	*Arctium pubescens*	–	31.0
	Elytrigia repens	–	29.7
	Galium aparine	–	39.0
	Heracleum sphondylium	–	23.3
	Urtica dioica	–	27.9
14. Fertilized meadow	*Bromus mollis*	–	29.8
	Dactylis glomerata	–	30.0
	Lolium perenne	–	32.5
	Ramunculus repens	–	29.8
	Taraxacum officinale	–	36.8
15. Reed marsh	*Cirsium vulgare*	–	19.4
	Eupatorium cannabinum	–	28.9
	Galium aparine	–	47.4
	Phragmites australis	–	23.5
	Urtica dioica	–	28.1

Appendix 2. *Calculation of the contribution of various chemical compounds to a difference in the construction costs of plants*

We assume that the chemical composition of a plant can be fully characterized by eight classes of compounds: minerals, organic acids, TNC, TSC, soluble phenolics, protein, lignin and lipids. For each of these classes of compounds (i), the specific cost of construction (S_i) has been estimated, with S_i being the amount of glucose (in g) required to construct 1 g of a given compound, with glucose and minerals as the starting point. For plants, this area was pioneered by Penning de Vries *et al*. (1974). A recent review is given by Poorter & Villar (1997). If the concentration (C) of each class of compounds (i) is known (C_i, given in g g^{-1}), then the construction cost (K) of a plant or plant organ is given by

$$K = \sum_{i=1}^{8} S_i C_i \qquad \text{Eqn A1}$$

Given a difference in chemical composition due to environment or species, the question then arises how we can quantify the effect on K. In the simple case where a plant consists of only two compounds, x and y, and that we consider only two environments, low (L) and high (H), respectively, the construction costs K^L and K^H are given by

$$K^L = C_x^L S_x + C_y^L S_y \qquad \text{Eqn A2}$$

and

$$K^H = C_x^H S_x + C_y^H S_y \qquad \text{Eqn A3}$$

Consequently, the difference in construction costs is

$$K^H - K^L = C_x^H S_x + C_y^H S_y - C_x^L S_x - C_y^L S_y \qquad \text{Eqn A4}$$

which can be rewritten as

$$K^H - K^L = (C_x^H - C_x^L) S_x + (C_y^H - C_y^L) S_y \qquad \text{Eqn A5}$$

This equation correctly calculates the exact difference in construction costs, but the two composing terms do not adequately describe the exact magnitude and direction of the change. To this end, we have to consider the specific construction costs (S) of each class of compound relative to the construction costs of the plant (or organ). How can we introduce that mathematically?

As the concentration of all compounds together is always 1, and there are only two compounds, x and y, it should be the case that

$$(C_x^H - C_x^L) + (C_y^H - C_y^L) = 0 \qquad \text{Eqn A6}$$

and also that

$$(C_x^H - C_x^L) K^L + (C_y^H - C_y^L) K^L = 0 \qquad \text{Eqn A7}$$

Subtracting this from the right-hand side of Equation A5 yields, after some rearranging

$$K^H - K^L = (C_x^H - C_x^L)(S_x - K^L) + (C_y^H - C_y^L)(S_y - K^L) \qquad \text{Eqn A8}$$

Each of these terms gives us the difference in construction costs due to the difference in composition between the L and H plants, using the construction costs of the L plants as a baseline. This approach can be easily extended from two to more compounds.