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Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems

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Summary

- The construction costs (*CC*) are reported of leaves from 162 wild woody species from 14 contrasting environments (desert to rain forest) and with different leaf life-spans.
- Calorimetric methods were used to estimate the *CC* of deciduous, semideciduous and evergreen leaves.
- Leaf *CC* showed a wide range (78%) between species, and deciduous species showed a slightly lower *CC* (6%) than both semideciduous and evergreen species. Mean leaf *CC* differed between ecosystems, with the highest and lowest *CC* in the tundra and rain forest, respectively. Leaf *CC* was positively correlated with lipid concentration. Leaf size (log) and specific leaf area (SLA, leaf area per leaf dry mass) were negatively correlated with leaf *CC*. Leaf *CC* did not show differences between different leaf life-spans or ecosystems when leaf size (log) or SLA were included as covariates.
- The small differences in leaf *CC* among leaf life-span types and ecosystems (6% and 23%, respectively) suggest that SLA is more important in determining differences in the carbon balance between species than leaf *CC*. Leaf size is shown to be an important trait associated with other leaf characteristics.

Key words: construction cost, deciduous, evergreen, heat of combustion, specific leaf area, lipids, nitrogen, leaf size.

Abbreviations

A, ash concentration; CC, construction cost per unit dry mass; CC_A, construction cost per unit area; Eg, growth efficiency; Hc, ash free heat of combustion; N, nitrogen; SLA, specific leaf area.

Introduction

Species with different leaf life-spans differ in many traits related to carbon flux, such as photosynthetic and respiration rates (Mooney & Gulmon, 1979; Field & Mooney, 1986; Reich *et al.*, 1992; Larcher, 1995; Villar *et al.*, 1995; Reich *et al.*, 1997). Fast growing species from favourable habitats show short leaf life-span and have high photosynthetic and respiration rates per unit mass (Poorter *et al.*, 1990; Reich *et al.*, 1992; Atkin *et al.*, 1996; Reich *et al.*, 1997). These differences could help explain the advantages of the different leaf life-spans in different habitats, and thus the distribution of species with different leaf longevity. However, to fully elucidate the advantages in terms of carbon balance of different leaf life-spans in different habitats, we also need to know the energetic costs of tissue construction (i.e. the construction cost, *CC*). The construction cost includes the glucose required to build carbon skeletons, and the glucose consumed in respiration to supply reductant and ATP for energy-requiring processes in the biosynthesis of the tissue constituents (Penning de Vries *et al.*, 1974; Williams *et al.*, 1987).

Although differences in leaf *CC* among species with different leaf life-span (evergreen and deciduous) have been studied for about three decades, no clear picture has emerged. Early studies suggested that evergreen leaves have higher *CC* than deciduous ones (Johnson & Tieszen, 1976; Orians & Solbrig, 1977; Miller & Stoner, 1979), since the former are richer in defensive compounds (such as lignin and antiherbivore compounds), which are expensive to synthesize (Mooney & Gulmon, 1979; Chabot & Hicks, 1982). Subsequent studies have supported this hypothesis (Merino, 1987; Diamantoglou *et al.*, 1989; Gower *et al.*, 1989; Damesin *et al.*, 1998) but others have not (Merino *et al.*, 1982; Chapin, 1989; Williams *et al.*, 1989).

Several factors could explain the contradictory measures of *CC* reported in the scientific literature. Firstly, relative estimates of *CC* will depend on the units with which they are expressed. For example, Sobrado (1991) found that evergreen leaves had higher *CC* per unit area (g glucose m^{-2}) than deciduous ones. However, recalculation of that data per unit dry mass (g glucose g^{-1}) showed that there were no significant differences in *CC* between these leaf types. Secondly, the size of the leaf seems to affect leaf *CC*. For example, Merino (1987) found higher leaf *CC* in evergreen than in deciduous leaves, but he did not find any difference between these leaf types when species with similar leaf size were compared. Thirdly, comparisons were often made between only two or three species. Moreover, in some cases, the species compared were native to different ecosystem types (tropical, mediterranean, arctic, etc.). Since, differences in resource availability, such as light, nitrogen and phosphorus, appear to affect *CC* (Griffin, 1994; Poorter, 1994; Griffin *et al.*, 1996; Poorter & Villar, 1997), we may expect ecosystem type to influence *CC*. Finally, because of the diversity of methods used to calculate *CC*, a comparison of the available data from different literature sources is unlikely to be a good method to test the hypothesis of whether *CC* differ among contrasting species and/or between contrasting environments. For example, comparisons of *CC* values obtained by different methods for the same plant material may differ by as much as 20% (Williams *et al.*, 1987; Griffin, 1994).

There is little information available on the differences in *CC* among species from contrasting ecosystems. A major focus of our study was therefore to assess whether leaf *CC* differ among species with different leaf life-span, and/or among species from contrasting ecosystems. The aims of this study were to test (1) if species with long leaf life-span have relatively high leaf *CC*, and (2) if mean leaf *CC* differs between contrasting ecosystems. The relationships between leaf *CC* and other leaf traits (size, chemical composition, etc.) were also investigated.

Materials and Methods

Different leaf traits of 162 wild woody species from 14 contrasting ecosystems (Table 1) were studied. Included in this sample, were published data on seven tropical dry forest species in South America (Sobrado, 1991) and 35 species from two rain forests in Africa (Waterman *et al.*, 1980). Both studies followed the same approach as in the present study. The species were classified according to the mean leaf life-span into the following categories: deciduous (4–8 months), semideciduous (5–12 months) and evergreen (> 12 months). Semideciduous species are those that have a leaf longevity of less than 1 yr, but in contrast to deciduous species, keep leaves throughout the year. Data on leaf life-span were taken from

Table 1 Type of the ecosystems studied, latitude and longitude, location and the number of deciduous (Dec), semideciduous (S-Dec) and evergreens species (Ever) considered in each ecosystem. Code of ecosystems used is as in Fig. 2. Data of ecosystem 12 are from Sobrado (1991) and data of ecosystems 13 and 14 from Waterman *et al.* (1980)

Code	Ecosystem	Latitude and longitude	Location	Dec	S-Dec	Ever
1	Tundra	75° N 82° W	Devon Island, Canada	1	0	2
2	Desert	28° N 106° W	Chihuahua, USA	2	1	3
3	Xeric forest	28° N 17° W	Canary Islands, Spain	4	4	6
4	Chaparral	36° N 122° W	California, USA	1	2	3
5	Xeric mediterranean forest	37° N 6° W	Andalucía, Spain	0	11	9
6	Mesic mediterranean forest	36° N 122° W	California, USA	6	0	4
7	Mesic mediterranean forest	37° N 6° W	Andalucía, Spain	13	2	9
8	Temperate forest	44° N 80° W	Toronto, Canada	4	0	0
9	Warm temperate forest	35° N 80° W	North Carolina, USA	7	0	1
10	Austral forest	55° S 70° W	Tierra del Fuego, Argentina	2	0	5
11	Lauriphyll forest	28° N 17° W	Canary Islands, Spain	1	0	17
12	Tropical dry forest	10° N 67° W	Charallave, Venezuela	4	0	3
13	Rain forest	5° N 10° W	Douala-Edea Forest, Cameroon	1	0	20
14	Rain forest	0°, 32° W	Kibale Forest, Uganda	3	0	11

literature and from field observations. Nomenclature of the species agreed with the classification given in taxonomic texts for each region (Thomas, 1961; Branwell & Branwell, 1974; Porsild & Cody, 1980; Benson & Darrow, 1981; Moore, 1983; Valdés *et al.*, 1987; Petrides, 1988).

The sampling was done following the same protocol during summer of 1990 and 1991. For each species, several individuals were sampled, taking branches found in different positions of each individual. All the leaves present in each branch were sampled, excluding those with injuries. Leaf blade area was determined in one subsample either using an image analyser (Skye Instruments, Ltd.) or by making photocopies of leaves with paper of known specific weight and weighting the leaf images. Leaf samples were oven dried at 80°C until constant weight, ground and homogenized for subsequent analysis. Ash concentration was determined gravimetrically after combustion of the sample for 4 h at 500°C. Total organic nitrogen concentration was determined by Kjeldhal analysis. Protein concentration was estimated by multiplying nitrogen concentration by 6.25 (Merino *et al.*, 1984). Heat of combustion was determined with an adiabatic bomb calorimeter (Phillipson Gentry Instruments, Inc., USA) with correction for ignition wire melting (Phillipson, 1964).

Lipid concentration was determined in leaves of 43 species, most of them native from xeric and mesic mediterranean forests (Spain), austral forest (Argentina), and chaparral scrub (CA, USA). We also include the data on lipid concentration of seven species from tropical dry forest in Venezuela (Sobrado, 1991). Lipid concentration was obtained gravimetrically from soluble diethylether extracts (Allen, 1974).

Leaf *CC* (g glucose g^{-1}) was calculated using a formula based on the growth efficiency of the leaf tissue, heat of combustion and ash and nitrogen concentration of leaves according to Williams *et al.* (1987):

$$CC = [(0.06968\,Hc - 0.065)(1 - A) + 7.5k\,N/14.0067)]/Eg \qquad \text{Eqn 1}$$

where Hc is the ash free heat of combustion (kJ g^{-1}), A is the ash concentration (g g^{-1}), k is the oxidation state of the nitrogen source (+5 for nitrate or –3 for ammonium), N is the organic nitrogen concentration (g g^{-1}) and Eg is the growth efficiency. The value used in this study for Eg was 0.89 (Williams *et al.*, 1987). In the calculations, we assumed that the nitrogen source was nitrate for all the species, as it is the principal source of nitrogen that is available to higher plants under most field conditions (Taiz & Zeiger, 1991). However, there is a broad consensus that in some ecosystems, for example tundra, the main nitrogen source is ammonia, although tundra species can also use nitrate (Atkin *et al.*, 1993). So, in the case of tundra species, we also consider ammonia as the nitrogen source for calculation of leaf *CC*. Heat of combustion, ash, and nitrogen and lipid concentration were measured from two different samples obtained from the homogenized leaves for each species. In cases in which variation was higher than 5%, a triplicate sample was considered.

The cost of protein synthesis (g glucose spent in protein synthesis per gram of dry tissue) was calculated by multiplying the protein fraction in the tissue by the specific cost of protein synthesis [2.775 g glucose (g protein)$^{-1}$] (Poorter, 1994). The percentage of *CC* dedicated to protein synthesis was calculated as the ratio: (cost of protein synthesis/*CC*) * 100.

Statistical analysis of data

Statistics were performed using Statistica (StatSoft, 1996) and SPSS (SPSS, 1999). Differences in leaf traits were analysed with a non-parametric test (Kruskal–Wallis) with leaf life-span or ecosystem as class factor. Comparison of leaf traits between leaves with different life-spans (class factor) were made in two ways: (1) pooling all species from different ecosystems and (2) independently in each ecosystem with two or more species belonging to at least two of the three different leaf life-span classes (deciduous, semideciduous or evergreen). Note that most ecosystems studied did not have species belonging to the three leaf types considered, and also that in some ecosystems the majority of species belongs to only one leaf life-span category (Table 1 and Appendix 1).

To detect differences in leaf traits between contrasting ecosystems, a non-parametric test (Kruskal–Wallis) with ecosystem type as class factor was performed. In doing so, the differences in leaf *CC* between contrasting ecosystems could be affected by the dominant leaf life-span of the species in each ecosystem. Therefore, to check if leaf *CC* were affected by ecosystem type within each leaf life-span type, we performed a Kruskal–Wallis test (ecosystem type as class factor) on two data sets separately; one for deciduous species and the other for the evergreen ones. In this analysis, only those ecosystems with at least four evergreen species or four deciduous ones were considered. Species with semideciduous leaves were not included in the analysis due to the low numbers of species of this type (i.e. there were only two ecosystems with at least four species).

A general linear model was fitted to leaf *CC* data with ecosystem, leaf life-span and leaf area (log) or SLA as explanatory variables using maximum likelihood methods. Ecosystem and leaf life-span were introduced as factors (14 ecosystems, two classes of leaf life-span: deciduous and evergreen) and leaf area (log) or SLA as covariates.

Although leaf *CC* results from the values of three independent variables (heat of combustion, nitrogen and ash concentration) (Eqn 1), the importance of each one of these in explaining the value of leaf *CC* was unknown. We explored the sensitivity of leaf *CC* to changes in each component (*Hc*, *N* or ash concentration) keeping the other two components constant (similar approach as Griffin *et al.*, 1996). Mean values of *Hc*, *N* and ash concentration obtained from our data set were chosen as constant values, and sensitivities of estimates of *CC* were calculated on the basis of a change in a variable value of plus or minus two times its standard deviation. We calculated the percentage of change in leaf *CC* that was caused by increasing each one of the independent variables from $\bar{x}$ – 2*S.D. to $\bar{x}$ + 2*S.D., maintaining the other two variables constant.

All means are presented with ± standard deviation.

Results

Leaf *CC* of the 162 species ranged from 1.08 g glucose g^{-1} (*Chaetacme aristata*, rain forest, Uganda) to 1.92 g glucose g^{-1} (*Erica scoparia*, mesic mediterranean forest, Andalucía, Spain) (see Appendix 1), with the mean leaf *CC* for all species being 1.52 ± 0.12 g glucose g^{-1}.

Causes of variation in leaf *CC*

Both the sensitivity of *CC* to small changes in variable value and the actual variation in parameter values contribute to the relative importance of each parameter in determining variation in *CC*. For example, *CC* was shown to be sensitive to small changes in *Hc*. However, there was very little variation in this measure between samples (C.V. = 6%), and therefore *Hc* contributed less than expected to the observed variation in *CC*. Contrary to this, *CC* was not very sensitive to changes in ash concentration, but this measure showed considerable variation between samples (C.V. = 50%) and therefore it determined more of the variation in *CC* than expected on the basis of its sensitivity (Fig. 1a). In any case, the most important parameter was shown to be *Hc*. The sensitivity analysis of leaf *CC* showed that increasing *Hc* from $\bar{x}$ – 2*S.D. to $\bar{x}$ + 2* S.D. caused an increase in leaf *CC* of 30%. The increase in ash concentration determined a decrease in leaf *CC* of 15%, whereas the increase in *N* showed the lowest effect on leaf *CC*, increasing about 6% (Fig. 1a).

Leaf *CC* was positively correlated with *Hc* ($r = +0.92$, $P < 0.0001$; Fig. 1*b*) and negatively correlated with ash concentration ($r = -0.62$, $P < 0.001$; Fig. 1*c*). However, leaf *CC* was not correlated with *N* ($r = -0.11$, $P > 0.17$; Fig. 1*d*).

Since *Hc* is the main determinant of the differences in leaf *CC*, it is worth investigating the parameters related to the variation in *Hc*. The value of *Hc* is determined by the chemical composition of the tissue (Williams *et al.*, 1987).

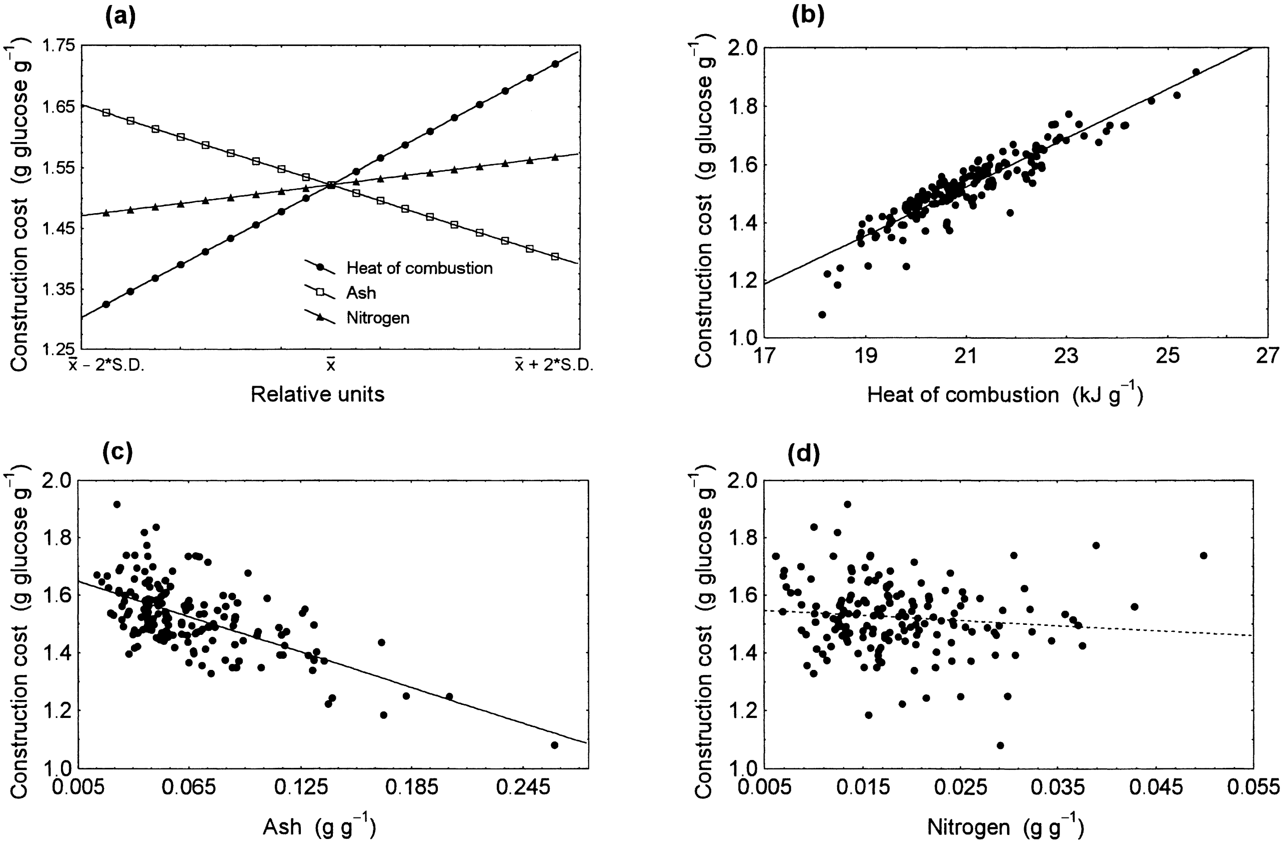

Fig. 1 (a) Sensitivity analysis of leaf construction cost to the increase or decrease in only one component (ash free heat of combustion, *Hc*; nitrogen, *N*; or ash concentration) keeping the other two components constant. Mean values of *Hc*, *N* and ash concentration of our data set were chosen as constant values and the amount of increase or decrease in the variables to detect its effect on leaf construction cost were ±2*S.D. Relationships between leaf construction cost (g glucose g^{-1}) and (b) ash free heat of combustion ($r = +0.92$, $P < 0.0001$), (c) ash concentration ($r = -0.62$, $P < 0.001$), and (d) nitrogen concentration of leaves (g g^{-1}) ($r = -0.11$, $P > 0.17$).

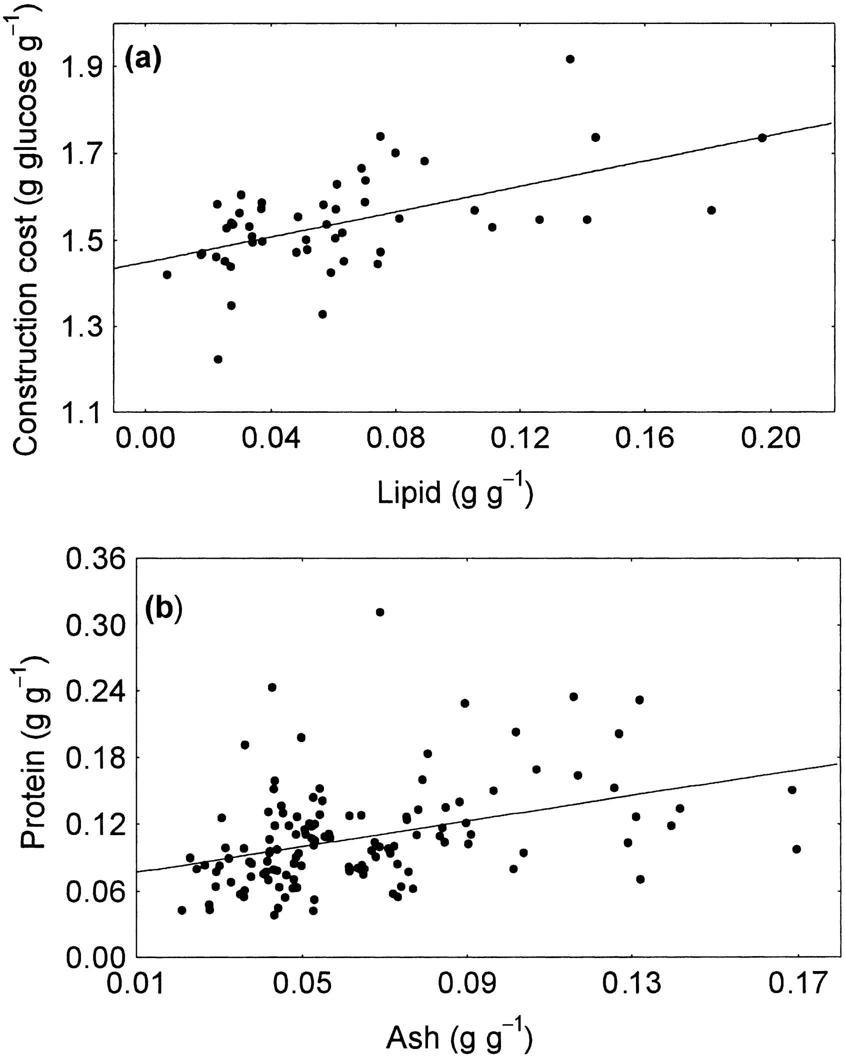

Fig. 2 Relationships between (a) leaf construction cost (g glucose g^{-1}) and lipid concentration (g g^{-1}) ($r = +0.54$, $P < 0.05$) and (b) protein concentration (g g^{-1}) and ash concentration (g g^{-1}) ($r = +0.38$, $P < 0.00001$).

We found a positive relationship between lipid concentration and both *Hc* ($r = + 0.61$, $P < 0.0001$) and leaf *CC* ($r = + 0.54$, $P < 0.05$, Fig. 2a). This suggests that lipid concentration could be one of the main factors responsible for the observed differences in leaf *CC* associated with leaf life-span and ecosystem type.

Proteins, which are one of the most expensive compounds to synthesize were positively correlated to minerals ($r = +0.38$, $P < 0.00001$, Fig. 2b), which have a null construction cost. Therefore, leaves with higher protein concentration have relatively high concentrations of minerals, which tends to keep *CC* values close to average *CC*.

Leaf *CC* between different leaf life-span and ecosystem type

Mean leaf *CC* of deciduous species (1.46 ± 0.12 g glucose g^{-1}) was significantly lower (6%; $P < 0.05$) than those of semi-deciduous and evergreen species (1.55 ± 0.10 and 1.55 ± 0.12 g glucose g^{-1}, respectively) (Fig. 3a). There were no differences in leaf *CC* between semideciduous and evergreen species. Leaves of evergreen and semideciduous species showed a higher heat of combustion, a lower nitrogen and ash concentration and a smaller leaf size than those of deciduous species ($P < 0.05$, Table 2). Deciduous species also show a significantly higher proportion of the leaf *CC* dedicated to protein synthesis (26%, $P < 0.001$) than evergreen and semideciduous species (19.6 and 17.5%, respectively) (Table 2).

The analysis of differences in leaf *CC* between deciduous and evergreen species within each ecosystem shows that only in the case of xeric forest (Canary Islands, Spain) and rain forest (Uganda), were evergreen leaves more costly to construct than deciduous leaves ($0.05 < P < 0.10$). No differences between *CC* of evergreen and deciduous leaves were found in the five other ecosystems where evergreen and deciduous were present (Table 1).

We found significant differences ($P < 0.0001$) in leaf *CC* between ecosystems (Fig. 4a) that were mirrored by significant

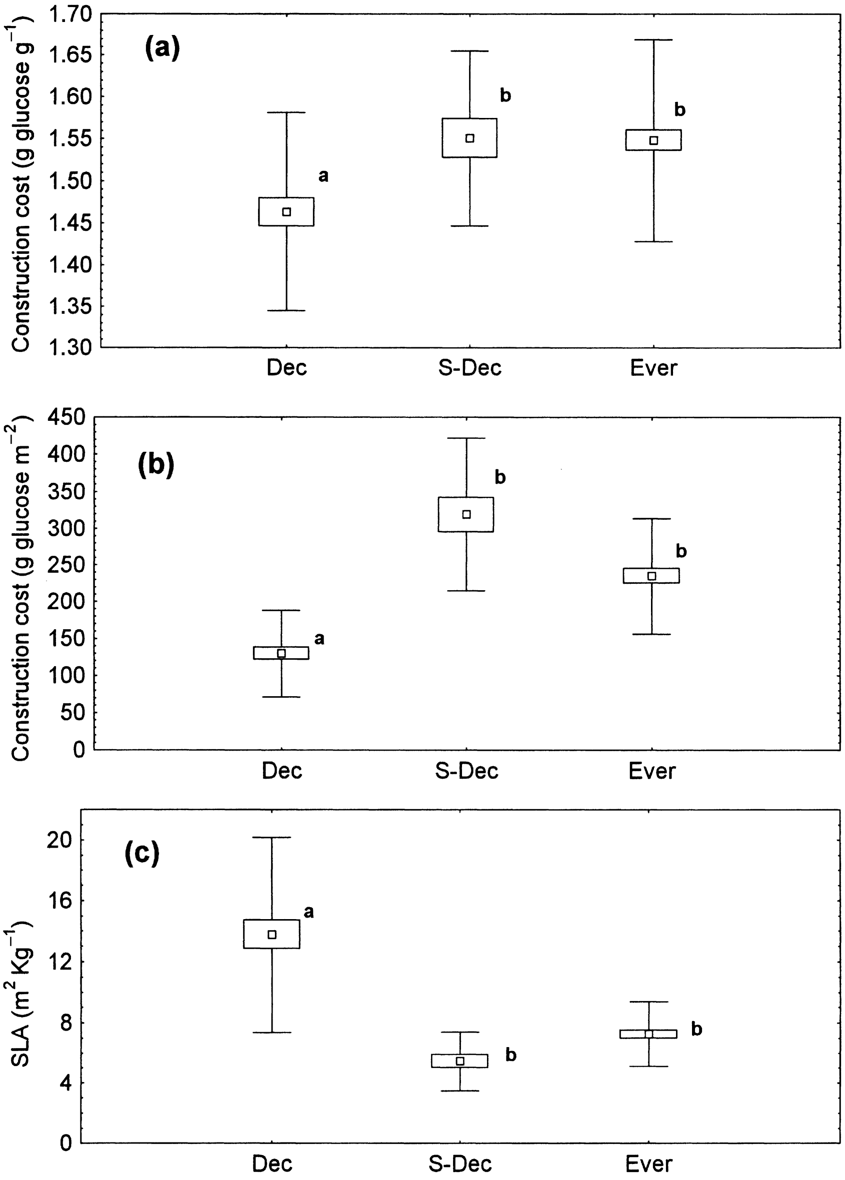

Fig. 3 (a) Mean leaf construction cost expressed per unit dry mass (g glucose g^{-1}), and (b) per unit area (g glucose m^{-2}), and (c) specific leaf area (SLA, m^2 kg^{-1}) in relation to the life span of the leaves (Dec, deciduous; S-Dec, semideciduous; and Ever, evergreen). Box limits correspond to ± SE and bars to ± SD. Different letters mean a significant difference ($P < 0.05$).

Table 2 Mean values (± SD) of ash free heat of combustion (*Hc*), nitrogen and ash concentration, leaf size and the proportion of construction cost dedicated to protein synthesis (CC proteins, [glucose used in protein synthesis/construction cost]*100) in leaves with different life span (deciduous, semideciduous and evergreen) from the 14 ecosystems considered (Table 1). In brackets, number of species considered. For leaf size the number of species considered were 41,19 and 56 for deciduous, semideciduous and evergreen, respectively. Different letters in one column means a significant difference ($P < 0.05$)

	Hc (cal g^{-1})	Nitrogen (mg g^{-1})	Ash (mg g^{-1})	Leaf size (cm^2)	CC proteins (%)
Deciduous ($n = 49$)	20.40 ± 1.03a	22.04 ± 7.7a	84.3 ± 48.9a	96.9 ± 373.5a	26.2 ± 9.0a
Semideciduous ($n = 20$)	21.32 ± 1.34b	15.65 ± 5.2b	57.9 ± 18.5b	3.0 ± 3.3b	19.6 ± 6.2b
Evergreen ($n = 93$)	21.24 ± 1.41b	17.30 ± 7.4b	59.0 ± 30.3b	16.6 ± 21.08c	16.2 ± 6.2b

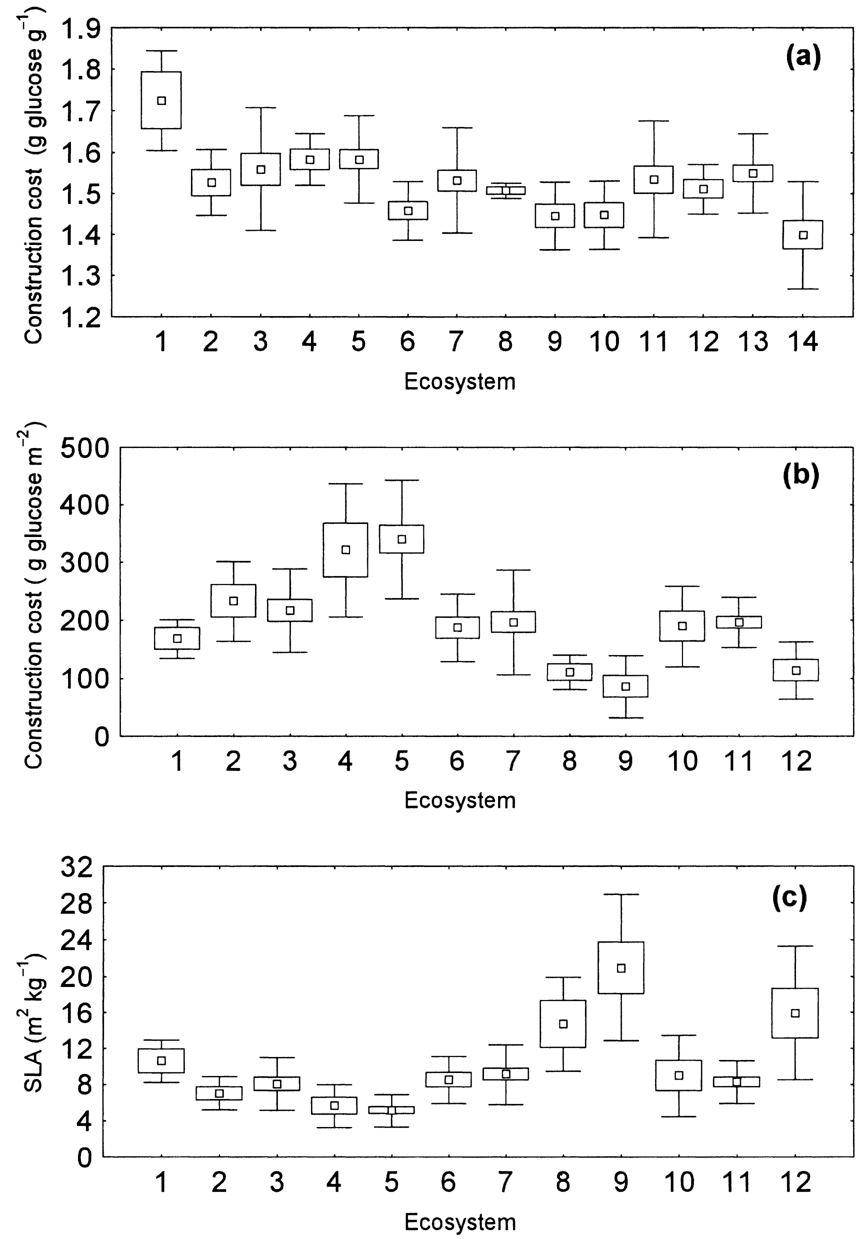

Fig. 4 (a) Mean leaf construction cost expressed per unit dry mass (g glucose g^{-1}), and (b) per unit area (g glucose m^{-2}), and (c) specific leaf area (SLA, m^2 kg^{-1}) of the species from 14 contrasting ecosystems. Ecosystem code as in Table 1. Box limits correspond to ± SE and bars to ± SD. No data are available for SLA in ecosystems 13 and 14.

differences in *Hc* ($P < 0.001$). Leaves of tundra species showed the highest *CC* (1.72 ± 0.11 g g glucose g^{-1}) whereas the lowest leaf *CC* correspond to species from rain forest (Uganda) 1.40 ± 0.13 g g glucose g^{-1}. When ammonia was assumed to be the principal nitrogen source in tundra species, the mean leaf *CC* was still higher than in other ecosystems (1.60 ± 0.16 g g glucose g^{-1}). The maximum difference in mean leaf *CC* between ecosystems was 0.32 g glucose g^{-1}, corresponding to about a 23% difference (0.32/1.40). However, when excluding the tundra species because of their low representation (only three species harvested), the ecosystems with highest leaf *CC* were xeric mediterranean forest (Andalucía, Spain) (1.58 ± 0.10 g glucose g^{-1}) and chaparral (1.58 ± 0.06 g glucose g^{-1}), and then the difference in mean leaf *CC* between ecosystems was much lower (13%), but still significant. We found a near significant correlation between mean leaf *CC* of each ecosystem and latitude ($P = 0.06$, $r = +0.51$), but excluding the tundra species there was no significant correlation ($P > 0.70$).

Deciduous leaves from different ecosystems did not show differences in *CC* ($P > 0.3$; range: 1.40–1.50 g glucose g^{-1}). In contrast, leaf *CC* of evergreen species were significantly different ($P < 0.001$) between ecosystems, with the highest values for the species from the xeric forest (Canary Islands, Spain, 1.66 g glucose g^{-1}) and xeric mediterranean forest (Andalucía, Spain, 1.63 g glucose g^{-1}). In these ecosystems

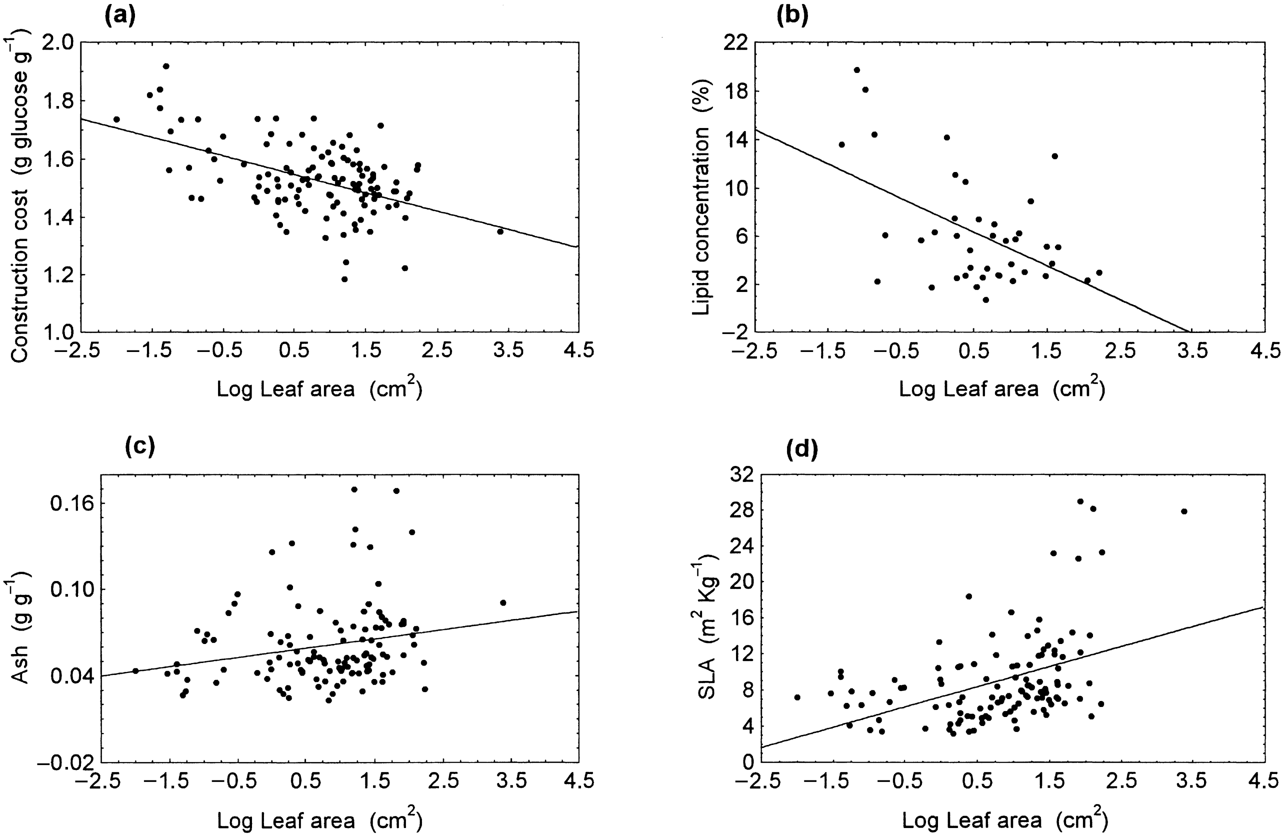

Fig. 5 Relationships between log leaf area (cm^2), and (a) leaf construction cost (g glucose g^{-1}) ($r = -0.42$, $P < 0.00001$), and (b) lipid concentration of leaves (g g^{-1}) ($r = -0.39$, $P < 0.01$), and (c) ash concentration (g g^{-1}) ($r = +0.22$, $P < 0.05$), and (d) specific leaf area (m^2 kg^{-1}, SLA) ($r = 0.43$, $P < 0.0001$).

Table 3 Results of two ways ANCOVA of leaf construction cost as dependent variable and leaf life-span (deciduous and evergreen) and ecosystem as main factors. In the analysis, log leaf area is included as covariate

Source	df	Sig.
Log leaf area	1	0.000
Leaf life-span	1	0.626
Ecosystem	10	0.133
Leaf life-span * Ecosystem	8	0.094

the evergreen leaves also showed a higher *Hc* ($P < 0.05$) than in the other ecosystems.

Leaf *CC* was negatively correlated with SLA ($r = -0.28$, $P < 0.0001$) and with the logarithm of leaf blade area ($r = -0.42$, $P < 0.00001$, Fig. 5a). SLA and leaf size showed a positive relationship ($r = +0.41$, $P < 0.00001$, Fig. 5d).

Taking into account the effect of leaf area when comparing the *CC* of leaves leaf life-span or ecosystem type were shown to have no significant effect (Table 3). Similar results were obtained when using SLA as covariate.

The logarithm of leaf size was negatively correlated with lipid concentration ($r = -0.39$, $P < 0.01$, Fig. 5b) and positively correlated with ash concentration ($r = +0.22$, $P < 0.05$, Fig. 5c).

Leaf *CC* per unit area and specific leaf area

Leaves with different life-span showed much larger differences in *CC* when expressed per unit area (CC_A) (130, 319, 237 g glucose m^{-2} for deciduous, semideciduous and evergreen species, respectively, $P < 0.00001$), than when *CC* was expressed per unit dry mass basis (Fig. 3a,b). These large differences in leaf CC_A (calculated as *CC*/SLA) were due more to the high differences in SLA (13.8, 5.5, 7.2 m^2 kg^{-1} for deciduous, semideciduous and evergreen species, respectively) (Fig. 3c) than to the differences in leaf *CC* per unit dry mass (Fig. 3a).

Similarly, mean leaf CC_A were significantly different ($P < 0.0001$) between ecosystems with values ranging from 86 g glucose m^{-2} (warm temperate forest, North Carolina, USA) to 321 g glucose m^{-2} in the chaparral (California, USA) (Fig. 4b). The differences in leaf CC_A between ecosystems were also mainly due to the differences in SLA, which ranged from 20.9 m^2 kg^{-1} in warm temperate forest species (North Carolina, USA) to 5.1–5.6 m^2 kg^{-1} in xeric mediterranean forest species (Andalucía, Spain) and chaparral (California, USA), respectively (Fig. 4c).

Discussion

Leaf *CC* between different species

Leaf *CC* of the species studied was in the range published for leaves of woody species from different ecosystems (Miller & Stoner, 1979; Merino *et al.*, 1982; Merino, 1987; Chapin, 1989; Sobrado, 1991). Studying young leaves, some authors (Merino *et al.*, 1984; Sobrado, 1994) obtained higher leaf *CC* than in our study (about 2.2 g glucose g^{-1}). The data in the present study refer to samples representative of all the leaf ages present in the plant (young, medium-aged and mature leaves). Thus, the results obtained represent a mean value of *CC* of the leaves of all age classes for each species.

Leaf *CC* showed a wide range between different species from 1.08 to 1.92 g glucose g^{-1}, which represents a 78% difference. Assuming similar differences in roots and stems, it could be significant for the carbon balance of a species, as individuals could grow expending 78% less energy than others; which could result, all things being equal, in higher relative growth rates or more energy allocated to defence and/or reproduction (Poorter & Villar, 1997).

Higher leaf *CC* results from higher *Hc* and lower ash concentrations (Fig. 1b,c). However, leaf *CC* is not positively correlated with *N* (Fig. 1d), perhaps due to the positive correlation between *N* (or protein concentration) and ash concentration (Fig. 2b). Because ash has a null direct cost (Penning de Vries *et al.*, 1974), a higher ash concentration is related to a lower *CC*. This explains the negative relationship of *CC* and ash concentration found in a previous study in tomato cultivars (Gary *et al.*, 1998) and in our study. *Hc* was positively correlated with lipid concentration, which explains nearly 40% of the variation in *Hc*. Similarly, Pantis *et al.* (1987) and Peng *et al.* (1993) found a positive correlation between lipid concentration and either *Hc* or *CC*. Lipids are one of the components with the highest energy content per unit mass and are one of the most expensive compounds to synthesize (3.030 g glucose g^{-1}, Penning de Vries *et al.*, 1974). In contrast, Poorter & Bergkotte (1992) did not find any relationship between lipid concentration and leaf *CC* in herbaceous species from central Europe, which could result from the low lipid concentration in these species (Poorter & Villar, 1997). Other compounds with high specific cost, such as lignin or phenols, could also explain the higher *CC* in some species.

Leaf *CC* between species with different leaf life-span

In contrast to the large differences in leaf *CC* between species (78%), the difference in mean leaf *CC* between leaf life-span types was small (6%, Fig. 3a). Differences in mean relative growth rate between deciduous and evergreen are much higher (98 and 15 mg g^{-1} d^{-1}, respectively) (Reich, 1998). The small difference observed in leaf *CC* between species with different leaf life-spans is therefore probably unimportant in determining differences in carbon balance between these groups.

Higher leaf *CC* in evergreen and semideciduous species result from their higher *Hc* and lower ash concentration (Table 2). The higher values of *Hc* in evergreens and semideciduous are caused by their higher lipid concentrations and possibly other compounds such as lignin or phenols (Poorter & Villar, 1997). Evergreen and semideciduous species from mediterranean ecosystems usually have a thick cuticle (Lillis, 1992), which is also found in species with low SLA (evergreens and semideciduous) from other ecosystems (Turner, 1994). The cuticle is rich in lipid compounds, which may be involved in reducing water loss (Larcher, 1995).

However, the differences between deciduous and evergreen leaves are not straightforward. First, the size of the leaves seems to play some role affecting leaf *CC* as small leaves have higher *CC* than large leaves (Fig. 5a). Because evergreen and semideciduous leaves considered in the present study have a smaller size than deciduous leaves (Table 2), leaf area could confound the effect of leaf life-span on *CC*. In fact, when leaf size (log) was included as a covariate, there was no significant difference in *CC* between leaf life-span types (Table 3). This result agrees with those of Merino (1987), who did not find any difference between leaf *CC* in deciduous and evergreen species when leaves of similar size were compared. In the present study, smaller leaves showed a low SLA (Fig. 5d) and have a higher lipid concentration (Fig. 5b). Smaller leaves are better suited in water-limited habitats since they have lower water requirements for temperature control (Gates, 1976). In addition, thicker leaves and cuticles reduce water expenditures, the latter generating higher *CC* (waxes and lipids are expensive to synthesize). Larger leaves require more structural support (i.e. more veins) (Niinemets & Kull, 1999), so they need to allocate more biomass to structural compounds, such as hemicellulose and cellulose, which have low specific costs. Large leaves also showed a high ash concentration (Fig. 5c), which could in part contribute to their lower leaf *CC*. Therefore; the higher leaf *CC* of evergreen and semideciduous species could be a consequence of their smaller size (or low SLA) 'per se', independently of their leaf life-span.

Secondly, the relationship between leaf *CC* and leaf life-span types is even less robust when we include semideciduous leaves in the comparison, as they show a life span similar to deciduous leaves (5–12 and 4–8 months for semideciduous and deciduous, respectively) and a *CC* similar to evergreen ones.

Finally, when we compare leaf *CC* of deciduous species with that of evergreen species within each ecosystem, we only found significant differences in two of the seven ecosystems compared.

Considering our results, there seems to be no clear relationship between leaf *CC* and leaf life-span.

Leaf *CC* between different ecosystems

The difference in leaf *CC* between ecosystems (about 23%) was higher than the differences in leaf *CC* between different leaf life-spans (about 6%). The higher leaf *CC* in different ecosystems was also related to higher *Hc*, which may be due to higher lipid concentrations. Lipids can play an important role in energy storage in plants from cold climates such as tundra (Bliss, 1962) or alpine ecosystems (Pantis *et al.*, 1987), which may explain the high leaf *CC* we observed in tundra species (Fig. 4a). Other differences in chemical composition, such as lignin or phenols, may also affect leaf *CC*.

Different hypotheses have been postulated regarding the effect of habitat on *CC* or *Hc*. Golley (1961) hypothesized that *Hc (*and thus leaf *CC*) should increase with latitude. The results of some studies agree with this hypothesis (Bliss, 1962; Adamandiadou *et al.*, 1978; Siafaca *et al.*, 1980). In our study, we also found a positive trend between leaf *CC* and latitude, but this pattern was lost when tundra species were excluded. Other authors (Penning de Vries *et al.*, 1974; Amthor, 1989) have suggested that the *CC* should be higher in stressful habitats. In contrast, Pitelka (1978) hypothesized that in stressful habitats, the energetic investment in the construction of one organ should be minimized to enhance fitness, resulting in lower *CC*. Our results do not support any of these hypotheses: species in stressful habitats (tundra and desert) showed high and intermediate leaf *CC*, respectively. Similarly, Merino (1987) did not find any pattern related to leaf *CC* in a set of 16 species growing in habitats differing in the degree of water and nutrient availability. One explanation is that in stressful habitats the individual plants could compensate either for higher, or lower, leaf *CC* by changes in their leaf biomass. Also, different types of stress could have different effects on leaf *CC*. Thus, a decrease in nitrogen availability appears to decrease leaf *CC* (Laffite & Loomis, 1988; Griffin *et al.*, 1993), while a decrease in phosphorus availability (Peng *et al.*, 1993), or an excess in light availability, appears to increase leaf lipid concentration (Loveless, 1961) and corresponding values for leaf *CC* (Williams *et al.*, 1989; Niinemets, 1999). The contrasting effects of different environmental factors on *CC* as mentioned above, could be one of the reasons for the lack of a clear pattern in leaf *CC* between ecosystems.

Also, it is important to consider that the pattern in leaf *CC* between ecosystems could be affected by the leaf life-span dominant in each particular ecosystem (i.e. in some ecosystems only one type of leaf life-span was found). Thus, the best way to compare ecosystems is to focus on one particular type of leaf life-span. It is remarkable that deciduous leaves showed similar *CC* in different ecosystems around the world, while evergreen leaves from two of the ecosystems considered (xeric forest of Canary Islands and xeric mediterranean forest in Spain) have higher *CC* than the evergreen leaves from all the other ecosystems. The former could be due to the fact that the environmental conditions in habitats where deciduous species are present, are quite similar (at least during the time of the year when the deciduous leaves are present).

Importance of leaf *CC*, chemical composition and leaf structure

The similarity of mean leaf *CC* between leaves with different life-spans and from different ecosystems is surprising. This similarity of mean *CC* cannot be due to the inability to measure differences in *CC*, because a wide range of leaf *CC* was found between species.

The positive correlation between components with high and low cost of synthesis, for example, that between proteins and minerals (Chapin, 1989; Poorter, 1994; Poorter & Villar, 1997, Fig. 2b), or the negative correlation between expensive compounds, for example, protein and lignin, lignin and tannin (Chapin, 1989), or protein and wax (Martínez *et al.*, unpublished), could explain the observed similarity in mean leaf *CC* in species with different leaf life-span or from different ecosystems. This means that a very different chemical composition might result in a similar leaf *CC* making leaf *CC* insensitive to differences in ecosystem or leaf life-span. For this reason, it may more adequate to consider how the energy is allocated in the leaf, more than the leaf *CC* value itself. A plant can allocate the energy to construct a leaf, either synthesizying different compounds, thus having a different chemical composition, and/or having a different leaf structure [i.e. changing the ratio leaf area (leaf dry mass)$^{-1}$, SLA]. Species with different leaf life-spans differ markedly in both aspects. Species with short leaf life-spans (deciduous) show a higher nitrogen concentration than evergreens (Cornelissen *et al.*, 1997, Table 2) suggesting that a higher fraction of the energy allocated to leaf constrution is used for protein synthesis (Table 2). Deciduous leaves are also characterized by a high SLA (Fig. 3c). Both variables, leaf nitrogen concentration (or protein concentration) and SLA are positively correlated to leaf photosynthesis (Field & Mooney, 1986; Reich, 1993; Reich *et al.*, 1997).

Leaf life-span has been found to be positively related to the ratio *CC*/daily C gain (Williams *et al.*, 1989) or *CC*/A_{max} (Sobrado, 1991), being A_{max} the maximum photosynthetic rate. This ratio (*CC* : C gain) is called payback time, which is defined as the time required for a leaf to fix the carbon necessary to equal the cost of leaf construction (leaf *CC*). Since Williams *et al.* (1989) and Sobrado (1991) did not find any difference in leaf *CC* between deciduous and evergreens, we can assume that the positive relationship between leaf life-span and payback time was mainly due to differences in C gain between the two types of leaves, more than to any relationship with *CC*. In fact, a general negative relationship of net photosynthesis and leaf life-span has been found for a wide range of species from different ecosystems (Kikuzawa, 1991; Reich, 1993; Larcher, 1995; Reich *et al.*, 1997). This suggests that leaf life-span seems to be more related to carbon gain than to energy spent in the construction of the leaf. The higher carbon gain of species with leaves of short life-span is then related to high leaf *N* and high SLA. As leaf *N* and SLA are highly correlated (Reich *et al.*, 1992; Reich *et al.*, 1997, our data set), and the range in SLA is larger between contrasting species than leaf *N* range, it follows that SLA comes up as the main single variable associated with differences in leaf longevity. In fact, SLA has been considered a key variable to explain differences in leaf life-span and relative growth rate in wild species (Lambers & Poorter, 1992; Atkin *et al.*, 1996; Cornelissen *et al.*, 1996; Poorter & van der Werf, 1998; Reich, 1998).

In summary, we conclude that leaf *CC* per unit dry mass showed large differences between species; lipid concentration (and probably, other chemical compounds) being the main factor responsible for these differences in leaf *CC*. However, functional groups (evergreen vs deciduous) and ecosystems showed small differences in leaf *CC*. These differences are very much higher when leaf *CC* is expressed in area basis, due to the large difference in SLA among species, functional groups and ecosytems. This suggests that SLA is a more critical variable associated with differences in leaf life-span and carbon balance between species, functional groups, and ecosystem types, than leaf *CC*.

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Appendix 1 Species studied, leaf construction cost (*CC*), specific leaf area (SLA) and leaf life-span (deciduous: D; semideciduous: S-D and evergreen: E). In the case of tundra species, the *CC* value calculated considering ammonia as nitrogen source is shown in brackets

Species	*CC* (g glu g^{-1})	SLA (m^2 kg^{-1})	Leaf life-span
TUNDRA *(Devon Island, Canada)*			
Cassiope tetragona	1.600 (1.497)	9.05	E
Dryas integrifolia	1.837 (1.789)	9.42	E
Salix arctica	1.737 (1.515)	13.27	D
DESERT *(Chihuahua, USA)*			
Artemisia tridentata	1.467	7.63	S-D
Chilopsis linearis	1.494	4.30	D
Chrysothamnus nauseosus	1.525	8.18	E
Flourensia cernua	1.538	8.63	D
Larrea tridentata	1.677	8.24	E
Simmondsia chinensis	1.462	5.08	E
XERIC FOREST *(Canary Island, Spain)*			
Adenocarpus foliolosus	1.775	10.02	E
Argyranthemum spp.	1.374	7.21	D
Bupleurum salicifolium	1.561	7.12	S-D
Chamaecytisus proliferus	1.740	6.59	S-D
Cistus monspeliensis	1.459	4.63	S-D
Cistus simphytifolius	1.475	6.05	S-D
Dafne gnidium	1.652	6.28	E
Dorycnium spectabile	1.515	12.42	D
Hypericum reflexum	1.506	9.13	D
Jasminum odouratissimum	1.715	6.47	E
Juniperus phoenicea	1.609	*n.a.*	E
Olea europaea	1.683	5.09	E
Pinus canariensis	1.515	*n.a.*	E
Rumex lunaria	1.244	10.71	D
CHAPARRAL *(California, USA)*			
Adenostoma fasciculatum	1.561	4.06	E
Arctostaphylos crustacea	1.609	5.29	E
Ceanotus cuneatus	1.490	3.60	E
Diplacus aurantiacus	1.638	8.33	S-D
Eriodictyon californicum	1.657	3.67	S-D
Lepechinia calycina	1.548	8.85	D
XERIC MEDITERRANEAN FOREST *(Andalucía, Spain)*			
Arctostaphylos uva-ursi	1.687	3.14	E
Cistus albidus	1.452	5.40	S-D
Cistus ladanifer	1.555	3.50	S-D
Cistus libanotis	1.582	3.69	S-D
Cistus monspeliensis	1.548	4.18	S-D
Cistus salvifolius	1.406	10.50	S-D
Halimium commutatum	1.463	3.40	S-D
Halimium halimifolium	1.467	6.07	S-D
Halimium umbellatum	1.570	3.56	S-D
Juniperus communis	1.629	6.66	E
Juniperus oophora	1.701	*n.a.*	E
Lavandula stoechas	1.735	6.30	S-D
Olea europaea	1.653	5.04	E
Phillyrea angustifolia	1.739	4.26	E
Phlomis purpurea	1.489	*n.a.*	S-D
Pinus pinaster	1.666	*n.a.*	E
Pistacia lentiscus	1.588	4.62	E
Quercus coccifera	1.532	6.04	E
Quercus rotundifolia	1.470	5.93	E
Rosmarinus officinalis	1.736	4.64	S-D
MESIC MEDITERRANEAN FOREST *(California, USA)*			
Aesculus californica	1.339	13.92	D
Alnus rhombifolia	1.463	10.33	D
Arbutus menziesii	1.480	6.97	E
Heteromeles arbutifolia	1.542	5.19	E
Quercus agrifolia	1.395	5.56	E
Quercus douglasii	1.450	7.91	D
Quercus keloggii	1.416	8.49	D
Quercus lobata	1.462	7.63	D
Rhamnus californica	1.437	10.68	E
Salix laevigata	1.596	8.25	D
MESIC MEDITERRANEAN FOREST *(Andalucía, Spain)*			
Arbutus unedo	1.536	6.48	E
Calluna vulgaris	1.736	7.11	E
Ceratonia siliqua	1.464	5.01	E
Cistus laurifolius	1.570	3.39	S-D
Cistus populifolius	1.446	4.85	S-D
Crataegus monogyna	1.505	10.63	D
Erica scoparia	1.917	6.18	E
Ficus carica	1.223	8.70	D
Frangula alnus	1.512	14.11	D
Fraxinus angustifolia	1.501	13.36	D
Fraxinus ornus	1.563	6.39	D
Mirtus communis	1.453	10.39	E
Nerium oleander	1.683	5.51	E
Populus alba	1.375	11.72	D
Pyrus bourgeana	1.572	11.81	D
Quercus faginea	1.528	9.17	D
Quercus lusitanica	1.537	6.76	D
Quercus pyrenaica	1.440	12.90	D
Quercus suber	1.541	7.07	E
Rubus ulmifolius	1.498	11.91	E
Salix sp.	1.583	9.36	D
Smilax aspera	1.605	8.48	E
Vitis vinifera	1.496	15.78	D
TEMPERATE FOREST *(Toronto, Canada)*			
Acer rubrum	1.488	22.56	D
Betula papyrifera	1.497	11.83	D
Populus tremuloides	1.526	12.30	D
Quercus rubra	1.519	12.10	D
WARM TEMPERATE FOREST *(North Carolina, USA)*			
Carya tomentosa	1.349	27.89	D
Chionanthus virginicus	1.443	28.95	D
Cornus florida	1.348	23.16	D
Ilex opaca	1.532	7.36	E
Liquidambar styraciflua	1.397	14.00	D
Liriodendron tulipifera	1.481	28.13	D
Platanus occidentalis	1.578	23.28	D
Populus heterophylla	1.435	14.34	D

Appendix 1 *continued*

Species	CC (g glu g^{-1})	SLA (m^2 kg^{-1})	Leaf life-span
AUSTRAL FOREST *(Tierra del Fuego, Argentina)*			
Berberis ilicifolia	1.421	4.87	E
Drimys winteri	1.479	6.85	E
Embothrium coccineum	1.518	7.82	E
Maytenus magellanica	1.329	7.32	E
Nothofagus antarctica	1.510	10.86	D
Nothofagus betuloides	1.531	6.65	E
Nothofagus pumilio	1.348	18.33	D
LAURIPHYLL FOREST *(Canary Islands, Spain)*			
Apollonias barbujana	1.493	7.07	E
Arbutus canariensis	1.490	6.97	E
Erica arborea	1.819	7.60	E
Erica scoparia	1.695	7.77	E
Heberdenia bahamensis	1.356	8.84	E
Ilex canariensis	1.567	6.34	E
Ilex platyphylla	1.535	7.13	E
Laurus azorica	1.563	7.76	E
Maytenus canariensis	1.184	8.47	E
Myrica faya	1.478	10.55	E
Ocotea foetens	1.643	9.10	E
Persea indica	1.574	8.41	E
Picconia excelsa	1.584	5.73	E
Prunus lusitanica	1.631	7.72	E
Rhamnus glandulosa	1.390	8.09	E
Salix canariensis	1.623	16.56	D
Viburnum rigidum	1.582	7.02	E
Visnea mocanera	1.413	7.19	E
TROPICAL DRY FOREST *(Charallave, Venezuela)*			
Beureria cumanensis	1.496	13.72	D
Capparis aristiguetae	1.473	8.90	E
Coursetia arborea	1.426	27.62	D
Curatella americana	1.574	11.49	E
Lonchocarpus dipteroneurus	1.550	23.92	D
Morisonia americana	1.589	8.80	E
Pithecellobium dulce	1.473	16.89	D

Species	CC (g glu g^{-1})	SLA (m^2 kg^{-1})	Leaf life-span
RAIN FOREST *(Douala-Edea forest, Cameroon)*			
Anthonotha gracilliflora	1.596	*n.a.*	E
Anthonotha macrophylla	1.611	*n.a.*	E
Barteria fistulosa	1.616	*n.a.*	E
Berlinia auriculata	1.545	*n.a.*	E
Cissus producta	1.429	*n.a.*	E
Coula edulis	1.669	*n.a.*	E
Deidamia clematoides	1.487	*n.a.*	E
Dichostemna caloneura	1.534	*n.a.*	E
Dichostemna glaucens	1.523	*n.a.*	E
Diospyros dendo	1.653	*n.a.*	E
Diospyros hoyleana	1.594	*n.a.*	E
Garcinia mannii	1.483	*n.a.*	E
Garcinia ovalifolia	1.696	*n.a.*	E
Leptaulus daphnoides	1.551	*n.a.*	E
Librevillea klainei	1.645	*n.a.*	E
Lophira alata	1.626	*n.a.*	D
Mammea africana	1.599	*n.a.*	E
Protomegabaria tapfiana	1.367	*n.a.*	E
Rauvolfia vomitoria	1.371	*n.a.*	E
Trichoscypha patens	1.528	*n.a.*	E
Uapaca staudtii	1.418	*n.a.*	E
RAIN FOREST *(Kibale forest, Uganda)*			
Bosquia phoberos	1.372	*n.a.*	E
Cassipourea ruwenzoriensis	1.494	*n.a.*	E
Celtis africana	1.249	*n.a.*	D
Celtis durandii	1.250	*n.a.*	D
Chaetacme aristata	1.082	*n.a.*	D
Dombeya mukole	1.443	*n.a.*	E
Erythrina excelsa	1.392	*n.a.*	E
Funtumia latifolia	1.564	*n.a.*	E
Markhamia platycalyx	1.472	*n.a.*	E
Milletia dura	1.559	*n.a.*	E
Pancovia turbinata	1.402	*n.a.*	E
Parinari excelsa	1.455	*n.a.*	E
Strombosia scheffleri	1.392	*n.a.*	E
Teclea nobilis	1.462	*n.a.*	E