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Differences in construction costs and chemical composition between deciduous and evergreen woody species are small as compared to differences among families

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ABSTRACT

We tested to what extent differences in construction costs (CC) and chemical composition of woody species are attributed to leaf habit. Eight evergreen and eight deciduous species belonging to six families were selected to form eight phylogenetic independent contrasts (PICs). The plants were grown from seed in a glasshouse. Differences in leaf, stem and root CC between evergreen and deciduous species were minor, the proportion of variance explained by leaf habit generally being less than 6%. Surprisingly, differences in leaf chemical composition between deciduous and evergreen species were small as well. Variation in CC and chemical composition among families was substantial, the factor 'family' explaining 50–85% of variance. We therefore conclude that in this case, phylogeny is a more important factor than functional group. Leaves of the fast-growing species in this experiment showed high levels of minerals, organic acids, proteins and lipids, whereas leaves of the slow-growing species had higher concentrations of soluble phenolics, lignin as well as higher carbon/nitrogen (C/N) ratio. These relationships suggest a trade-off between growth and defence. In contrast, CC of leaves, stems, roots or whole plants showed no or only a weak correlation with relative growth rate (RGR). The C/N ratio of the leaves is an easily measured parameter that correlated strongly in a negative way with the RGR of the plants and reflected better the balance between investment in structure and physiological functioning than CC.

Key-words: C/ N ratio; leaf habit; lignin; lipid; organic acids; phylogeny; protein; relative growth rate.

INTRODUCTION

In an effort to understand functional differences among plant species, the role of photosynthesis has received considerable attention. However, important differentiation may also occur downstream of the carboxylation process. Allocation of the fixed carbon to various organs of a plant is likely to be a key process, and so is the regulation of biosynthetic reactions that determine how sucrose is converted to different chemical compounds within each organ. As the biochemical pathways for most constituents are known, it can be calculated how much sugars next to N, P and S are required to build the different classes of organic compounds (Penning de Vries, Brunsting & van Laar 1974). Some classes of compounds, such as lipids, are very expensive, which means that they require relatively large amounts of sugars (> 3 g) to form 1 g of end product. Others, such as organic acids, are relatively cheap and it costs close to 1 g of sugars to synthesize 1 g of a compound. If the proximate chemical composition is known, the total amount of photosynthates required to build 1 g of biomass can be calculated. This entity is defined as the construction costs (CC) of a plant organ.

Compared to our knowledge of the process of photosynthesis, we only have limited understanding of intraspecific and interspecific variation in the CC of various plant organs. Most of the data obtained so far are from field studies where leaves of deciduous and evergreen species were compared. In a number of studies, the CC of leaves of the evergreen species were found to be 5–20% higher than those of the deciduous ones (Miller & Stoner 1979; Merino 1987; Williams, Field & Mooney 1989; Eamus & Prichard 1998; Villar & Merino 2001). The explanation given for these results is that long-lived leaves have higher CC as a result of a higher investment in costly structural and defence compounds such as lignin, tannin and soluble phenolics (Chabot & Hicks 1982; Williams *et al*. 1989; Kikuzawa 1991). Others, however, did not observe differences between both functional groups in such different ecosystems as tundra (Chapin 1989), tropics (Sobrado 1991) or the Mediterranean area (Navas *et al*. 2003). Merino, Field & Mooney (1984) even found higher CC for deciduous leaves than evergreens, be it that they studied only three species.

All the data mentioned before are from field experiments, where species comparisons are complicated because environmental conditions may vary between sites. This may

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confound the comparative approach, as it has been shown that differences in the plant's environment (N and P availability, CO_2, irradiance) may significantly affect CC (Lafitte & Loomis 1988; Griffin, Thomas & Strain 1993; Baruch & Goldstein 1999; Niinemets 1999; Poorter & De Jong 1999; Martinez *et al*. 2002; Nagel *et al*. 2004; Poorter *et al*. 2006). A better understanding of possible inherent variation in CC can be obtained by growing plants under similar, controlled conditions. In this paper, we focus on a range of glasshouse-grown woody species that differ in leaf habit (deciduous versus evergreen). The first aim of this paper was to analyse whether the differences in leaf construction costs between deciduous and evergreen species, as sometimes observed in the field, still show up if plants are grown under exactly similar conditions.

Seedlings of woody species generally have rather similar proportions of biomass invested in leaves, stems and roots as herbaceous plants (Poorter & Nagel 2000). However, when woody plants become larger, the leaves take up a continuously smaller proportion of the total biomass, with values being lower than 5% in adult trees (Körner 1994). This may have consequences for whole plant CC, if the CC of stems and roots differ from those of leaves. We only have very limited knowledge about the CC of these organs. Poorter & Bergkotte (1992) compared the CC of leaves, stems and roots of 24 herbaceous species, and found roots to be 15% cheaper to build than leaves. No systematic comparisons of CC for leaves, stems and roots were made for a range of woody species. Therefore, our second aim was to analyse how CC of stems and roots of woody species relate to those of the leaves. Moreover, we would like to test whether differences exist between deciduous and evergreen species in this respect.

In most analyses, CC are derived from relatively quick measurements, based on strong correlations between the CC of different organic compounds with either heat of combustion (Williams *et al*. 1987) or C concentration (Vertregt & Penning de Vries 1987). Such information does not provide insight into the actual causes underlying possible differences in CC. To this end, information is required about the chemical composition of a plant's organ. In this way, Chapin (1989) showed that the relative constancy of the CC in tundra plants was caused by the fact that the concentration of one class of expensive compounds (lignin) was correlated negatively with the concentration of two other classes of high-costing compounds (protein and tannin). Other studies (Poorter & Bergkotte 1992; Villar & Merino 2001) have found that the main reason for construction costs being so similar across species was the positive correlation between the concentration of an expensive compound (protein) and a cheap one (minerals). Very few analyses of the proximate chemical composition together with CC were carried out. To obtain a better insight into the causes of variation in CC of woody species, the third aim of this experiment was to determine the chemical composition of the leaves of the evergreen and deciduous species, and to relate these differences to the observed variation in CC.

As part of the same experiment described here, Antunez, Retamosa & Villar (2001) measured the relative growth rate (RGR) of 16 species. It turned out that there was a large overlap in RGR between the deciduous and evergreen species, although on average, deciduous species showed higher RGRs than evergreens. To place the current data in the wider perspective of a plant's C-economy, we analysed the chemical composition of leaves and CC of various organs as a function of RGR. Growth is the net result of the C gained in photosynthesis and the way it is spent in respiration and in the anabolic pathways. As such, it is interesting to know how fast- and slow-growing woody species build up their biomass and to analyse what the implications are for their CC.

As mentioned earlier, most of the studies on CC have focused on leaves, and very few on stems, roots or fruits. In the end, a more complete picture can only be obtained if we know the CC of a whole individual. This requires knowledge of both the CC and biomass allocation of a plant, information which is seldom collected concurrently. RGR can be factorized as $RGR = (P * LMF - R)/CC_w$, where P is the mass-based photosynthetic rate, LMF is the fraction of plant biomass allocated to leaves, R is the respiration rate for maintenance and uptake of nutrients and CC_w is the whole-plant CC (Poorter & Villar 1997). Following this approach, a negative relationship could be expected between RGR and CC_w. It was suggested that if there is a trade-off between growth rate and defence, one would expect slow-growing species to invest strongly in secondary compounds and therefore to have high CC (Herms & Mattson 1992). Indeed, some authors report a negative correlation between CC and RGR (Nagel *et al*. 2004), but others do not find this (Poorter & Bergkotte 1992). The fourth aim of this paper was to analyse CC_w and leaf chemical composition as a function of the plant's RGR, to test whether such a trade-off is present in these woody species.

As far as we know, none of the studies concerning the CC and chemical composition of deciduous and evergreens species has taken into account the phylogenetic relationship among the species. This can be important as differences observed between these functional groups of species could be related to the trait of interest (e.g. evergreen versus deciduous), but also be attributed to the fact that the species come from evolutionary different families. A way to control for this potentially confounding problem is the 'phylogenetic independent contrast (PIC)' approach (Harvey & Pagel 1991; Saverimuttu & Westoby 1996; Swanborough & Westoby 1996; Villar *et al*. 1998; Ruiz-Robleto & Villar 2005). Species are selected in such a way that within a taxon level, two species form a PIC. They differ in a given attribute and form one fork in the evolutionary tree, allowing for comparison of a given attribute within a PIC independent of the comparison in other PICs. In the present study, we test the association between leaf habit (deciduous and evergreen) and CC as well as chemical composition by employing a PIC-based comparative analysis, used both at the design stage and during data analysis (Armstrong & Westoby 1993).

In summary, the aims of this paper were: (1) to investigate the differences in leaf CC between deciduous and evergreen species; (2) to analyse how the CC of stems and roots of woody species relate to those of the leaves; (3) to understand the relationship between CC and underlying chemical composition; and (4) to relate CC and chemical composition to the RGR of evergreen and deciduous species.

MATERIALS AND METHODS

Growth conditions

Sixteen woody species (eight deciduous and eight evergreen ones) were selected for the experiment, such that there was a contrasting pair of species (PIC) for a range of different plant families (Table 1). The seeds were sown in 3.3 L pots filled with a mixture of sand and turf in a 3:1 ratio, with nutrients provided by way of a slow-release fertilizer (Compo Nitrophoska, BASF, with 12% N, 12% P_2O_5, 1.2% Mg, 6% S, 0.1% Mn, 0.05% B, 0.02% Zn and other micronutrients in smaller proportions). The pots were placed in a glasshouse, where water was provided everyday to field capacity. Temperature in the glasshouse varied between 19.5 and 34.2 °C during the experiment. Daily observed maximal photon irradiance was on average 1100 μmol m^{-2} s^{-1}. More details about germination and growth are provided by Antunez *et al*. (2001). Three harvests were made at weeks 12, 18 and 26 after the onset of germination, with leaves, stems and roots collected separately. Each harvest consisted of 9–13 individuals per species, with plant mass determined after drying at 70 °C for at least 48 h. For subsequent chemical analysis, a minimum amount of 750 mg was required per sample. Therefore, for each organ we combined, separately, material of 3–4 individuals from the three harvests. In this way, we obtained three independent replicates per organ and species, except for the small-seeded *Ficus retusa* for which we had only one mixed sample.

Chemical analyses

Chemical analyses followed the procedures described in Poorter & Villar (1997). In short, each plant sample was ground with a mill to pass an 80 μm sieve. Thereafter, the samples were redried at 60 °C for 24 h. All subsequent measurements were carried out for each of the three independent samples. C and N concentration were measured with a CHN analyser (Carlo Erba, Milano, Italy). Nitrate was determined colourometrically following Cataldo *et al*. (1975). Another subsample was combusted at 550 °C in a muffle furnace, with ash weight determined on the residue. The ash consists partly of minerals, partly of oxides that are derived from organic acids, and nitrate. On cooling, the oxides react with CO_2 to form carbonates. Total amount of carbonates is determined by ash alkalinity. The leaf samples were further processed by extracting lipids and soluble phenolics from a third subsample with a mixture of chloroform and methanol according to Bligh & Dyer (1959). Addition of water produced chloroform and methanol/water phases. The chloroform was evaporated with N_2 and the residue was weighed. This residue will largely contain phospholipids and galactolipids, as well as some sterols, and is termed 'lipids' throughout this paper. The water/methanol phase contains the soluble sugars (gluocose, sucrose, soluble fructan, etc.), which can be determined with the anthrone method. In the same phase are the soluble phenolics, which can be measured with the Folin–Ciocalteu reagent. The residue left after extraction with chloroform/methanol was boiled for 3 h with 3% HCL, to determine insoluble sugars. C and N contents of the residue left over after this analysis were used to estimate the concentration of lignin and total

Table 1. Family and species studied, and the code of the phylogenetic independent contrast (PIC) used in the graphs

Family	PIC code	Species	Leaf habit
Anacardiaceae	1	*Pistacia terebinthus* L.	D
		Pistacia lentiscus L.	E
Fabaceae	2	*Gleditsia triacanthos* L.	D
		Ceratonia siliqua L.	E
Fagaceae	3	*Castanea sativa* Mill.	D
		Quercus coccifera L.	E
	4	*Quercus pyrenaica* Willd.	D
		Quercus ilex ssp. *ballota* L. (Desf.)	E
	5	*Quercus robur* L.	D
		Quercus suber L.	E
Moraceae	6	*Ficus carica* L.	D
		Ficus retusa L.	E
Oleaceae	7	*Fraxinus angustifolia* L.	D
		Olea europaea ssp. *sylvestris* L.	E
Sterculiaceae	8	*Sterculia platanifolia* L. f.	D
		Sterculia diversifolia G. Don	E

Nomenclature follows Romero (1984) and Castroviejo *et al*. (1989).
Leaf habit: deciduous (D) and evergreen (E).

structural carbohydrates (TSC) (see Poorter & Villar 1997 for more details).

Calculations and statistics

Concentration of organic acids was derived from the ash alkalinity and nitrate concentration (see Poorter & Villar 1997 for more details). Mineral concentration was calculated by subtracting the weight of oxides from the ash, and adding the nitrate concentration. CC were estimated with the concentration of C, minerals and organic N, following the method of Vertregt & Penning de Vries (1987) as modified by Poorter (1994). Therefore, for each organ of each species, we calculated concentrations of C, N, minerals, organic acids and protein, as well as the carbon/nitrogen (C/N) ratio. For leaves, we could also calculate the concentrations of lipids, soluble phenolics, TNC (total non-structural carbohydrates: soluble plus insoluble sugars), TSC and lignin. Recovery was calculated as the sum of concentration of the eight classes of compounds (minerals, organic acids, protein, lipid, soluble phenolics, lignin, TNC and TSC), relative to the theoretical 1000 mg g^{-1}. Recovery is 100% if all determinations are correct and the eight classes represent all of the chemical compounds in the plant.

To calculate the effect of the difference in chemical composition between deciduous and evergreen species of each PIC on variation in leaf CC, we followed the approach of Poorter & De Jong (1999). In this calculation, a given compound can cause variation in CC when the two species differ strongly in the concentration of that compound, or when that specific compound has glucose costs that are deviating strongly from the average plant CC:

$$CC_x^D - CC_x^E = ([X^D] - [X^E]) * (S_x - CC^E) \quad (1)$$

where CC_x indicates the CC for compound 'x'; [X] is the concentration of compound 'x'; S_x indicates the specific glucose costs for that compound; and CC^E is the total CC of the evergreen leaf of each PIC. The super indices 'D' and 'E' refer to the deciduous and evergreen species of each PIC. The specific glucose costs for each compound were taken from Poorter & Villar (1997).

Data were statistically analysed with Statistica (StatSoft 1996), using the procedures 'analysis of variance (ANOVA)' as well as 'correlations'. To take into account the phylogenetic relationships among the species, we considered 'Family' as a random factor and 'Leaf habit' (deciduous or evergreen) as a fixed factor in the ANOVA, similarly to Hoffmann & Franco (2003). When necessary, the data were transformed to log or arc sin to fulfil the assumptions of ANOVA. Another test, the non-parametric Fisher exact test, was performed to compare the chemical composition in the evergreen and deciduous members of each PIC. In most cases, the results of the Fisher exact test were equal to the ANOVA, showing that the results were rather robust. For correlations with RGR and specific leaf area (SLA) we used the data of Antunez *et al*. (2001). Principal component analysis (PCA) was carried out with SPSS (version 10).

RESULTS

Construction costs

Leaf CC, as calculated from C, organic N and mineral content, ranged from 1.21 g glucose g^{-1} for *F. retusa* to 1.65 g glucose g^{-1} for *Gleditsia triacanthos*, but the coefficient of variation for the 16 species was very small (8%) (Appendix I). There was no significant difference between the CC of evergreens and deciduous species (Fig. 1a; $P > 0.2$) and consequently leaf habit explained very little (*c.* 1%) of the total variance (Table 2). The main factor responsible for differences in leaf CC was the family (83% of variance explained). Species belonging to the *Ficus* and *Sterculia* genera were those with lower CC, whereas the *Pistacia*, *Gleditsia*/*Ceratonia* and *Fraxinus*/*Olea* genera had the highest values (Fig. 1a). In fact, variation in CC within each functional group was much larger (up to 0.4 g glucose g^{-1}, Fig. 1a) than between the two functional groups.

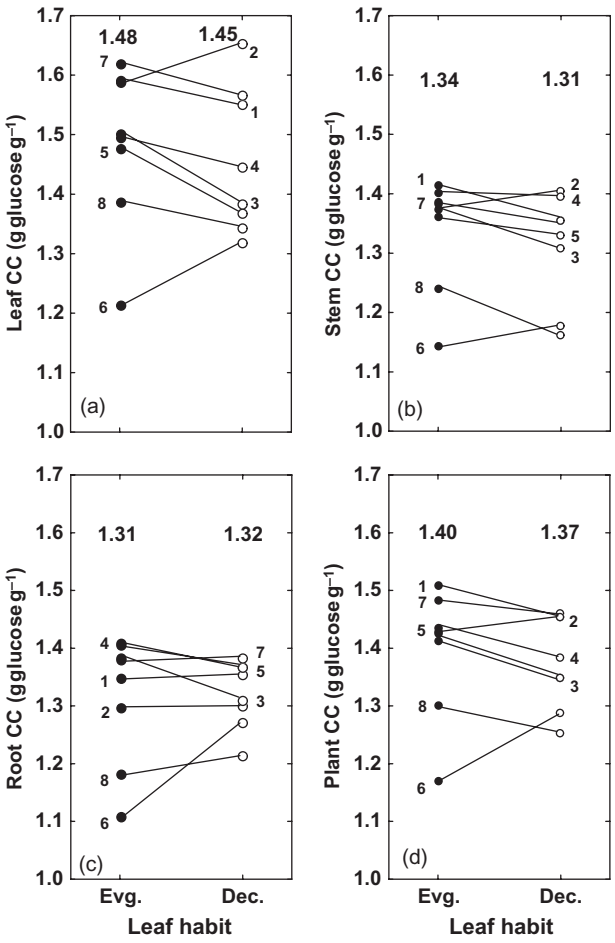

Figure 1. Construction costs (CC) for (a) leaves, (b) stems, (c) roots and (d) whole plants of evergreen (Evg.) (shaded circles) and deciduous (Dec.) (unshaded circles) species for the eight phylogenetic independent contrast pairs. Lines connect the points for each evergreen–deciduous pair (see Table 1 for codes of the pairs). Numbers in bold in each panel indicate mean values for evergreen and deciduous species.

As for leaves, the variation in CC of stems and roots was highly affected by family (77 and 80% of the variance explained, respectively), but not by leaf habit (1–2% of variance explained; Table 2, Fig. 1b & c). Similarly as in leaves, the coefficient of variation of CC for the 16 species was very small (7% for both stems and roots) (Appendix II). Families with the highest CC in leaves were also the ones with highest CC in stems and roots (Fig. 1 and Appendix II) and, consequently, with highest CC_w values (Fig. 1d and Appendix II). Averaged across species, CC for stems and roots were both 11% lower than for leaves (Table 3), with no significant differentiation between deciduous and evergreen species.

Chemical composition

A surprisingly small proportion of the variance (from <1–6%) in leaf chemical composition was explained by leaf habit, with differences generally being non-significant (Table 2). The only exception was the concentration of minerals, which was on average 38% higher for deciduous species (Fig. 2a). The Fisher exact test also found higher mineral concentration in deciduous species. Family, however, explained much more of the variance in chemical composition (51–86%). There were marked family differences for almost all classes of compounds (Fig. 2). Species from the *Ficus* genus had high concentration of minerals and organic acids (Fig. 2a & b), but had very low levels of lignin (Fig. 2f). Species from the *Ceratonia*/*Gleditsia* and *Ficus* genera had high protein concentration (Fig. 2c). High lipid concentrations were characteristic for species from the *Fraxinus*/*Olea* and *Ficus* genera (Fig. 2d), whereas species of the *Pistacia* and *Castanea*/*Quercus* genera had high concentrations of soluble phenolics (Fig. 2e). However, if we analyse the data without considering the family factor (a one-way ANOVA with leaf habit as factor), we can find statistical differences between deciduous and evergreen species in most compounds. In this case, deciduous species showed higher concentrations of minerals, organic acids and proteins, but had lower values for soluble phenolics and lignin than evergreens ($P < 0.05$).

Average recovery across species was 84%, but there were differences between families, with *Pistacia* species having low recovery values (74%) and species of *Ficus* showing the highest values (95%; data not shown). No difference in recovery between evergreens and deciduous species was found (Table 2).

Because the determinations are very time-consuming, we did not do a complete chemical analysis of stems and roots. However, as far as compounds were determined, we found similar results as for leaves. The main factor explaining the variance between chemical composition in stems and roots was the family (between 15 and 80%), and we did not find any significant differences between evergreen and deciduous species (only 1–6% of variance explained). The main difference with the results for leaves is that the protein concentration in roots was not significantly different between families and the percentage of variance explained was low (15%). There were generally highly positive correlations between the chemical composition of leaves and those of stems and roots (Table 3). However, stems and roots had lower concentrations of organic acids, carbon and protein than leaves and a higher C/N ratio (Table 3). Stems also had lower concentrations of minerals than leaves (Table 3).

Taken over all species, concentrations of leaf minerals, organic acids, protein and lipids were positively correlated with each other (Table 4). There were positive correlations between soluble phenolics and lignin as well. In Fig. 3, the PCA showed that some variables cluster together (which implies positive associations) whereas others are far from each other (which implies negative associations). We can distinguish two different groups of variables that were negatively correlated. The first group includes lipid, protein,

Table 2. Percentage of variance explained by the factors: 'Leaf habit', 'Family' and 'Leaf habit × Family', and the statistical significance of these factors using analysis of variance (ANOVA) with 'Leaf habit' as fixed factor and 'Family' as random factor

	Factor		
Variable	Leaf habit	Family	Leaf habit × Family
Leaf			
Minerals	6*	51***	2^{ns}
Organic acids	1^{ns}	69***	8*
Protein	3^{ns}	77***	6***
Lipids	$< 1^{ns}$	82***	2^{ns}
Soluble phenolics	3^{ns}	76***	8***
Lignin	6^{ns}	65***	8***
TNC	$< 1^{ns}$	86***	3^{ns}
TSC	1^{ns}	73***	7*
Carbon	2^{ns}	75***	5***
C/N ratio	3^{ns}	75***	6***
CC	$< 1^{ns}$	83***	8***
Recovery	$< 1^{ns}$	73***	4^{ns}
Stems			
Minerals	1^{ns}	57***	5^{ns}
Organic acids	6^{ns}	53***	21*
Protein	$< 1^{ns}$	48***	19**
Carbon	$< 1^{ns}$	71***	4^{ns}
C/N ratio	$< 1^{ns}$	44***	19**
CC	1^{ns}	77***	4^{ns}
Roots			
Minerals	$< 1^{ns}$	48***	3^{ns}
Organic acids	2^{ns}	43***	3^{ns}
Protein	$< 1^{ns}$	15^{ns}	2^{ns}
Carbon	$< 1^{ns}$	76***	7^{a}
C/N ratio	$< 1^{ns}$	28*	3^{ns}
CC	2^{ns}	80***	11**

Proportion of variance by factor '*x*' was calculated as SS_x/SS_{total} using the module 'Variance components' of Statistica 5.1. Some of the variables were log or arc sin transformed to fulfil the assumptions of ANOVA.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

[a]$0.05 < P < 0.10$; ns, non-significant.

TNC, total non-structural carbohydrates; TSC, total structural carbohydrates; C/N, carbon/nitrogen; CC, construction costs.

Table 3. Ratios of chemical composition and construction costs (CC) for stems relative to leaves; roots relative to leaves and stems relative to roots, and the correlation coefficient (r) for the chemical composition of the two organs

	Stems/Leaves		Roots/Leaves		Stems/Roots	
	Average ratio	r	Average ratio	r	Average ratio	r
Minerals	0.80*	0.71***	1.11^{ns}	0.20^{ns}	0.77**	0.36*
Organic acids	0.89***	0.48***	0.72***	0.54***	1.20*	0.25^{ns}
Carbon	0.96***	0.89***	0.95***	0.56***	1.01*	0.65***
Protein	0.51***	0.60***	0.62***	0.43**	0.81***	0.45**
C/N ratio	1.86***	0.69***	1.46***	0.53***	1.28***	0.53***
CC	0.89***	0.79***	0.89***	0.50***	1.00^{ns}	0.69***

Ratios are the back-transformed averages after logarithmic transformation. Signs in ratios indicate to what extent the average statistically deviates from 1.0 (= no difference between the concentration in the two organs considered). Signs in the correlation value indicate if the correlation is statistically significant.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$;
C/N, carbon/nitrogen.

minerals, organic acids and RGR. The second group includes lignin, soluble phenolics, C, C/N and CC. TNC and TSC are generally not correlated with the other variables (Table 4), so they appeared apart from the other two groups of chemical compounds (Fig. 3).

The relationships found among the concentrations of different chemical compounds tend to equalize the CC. Two types of relationships are found: (1) an expensive compound (protein) was positively related to a cheap compound (minerals) (Table 4; Fig. 3); and (2) one expensive constituent (protein) was negatively related to other expensive constituents (lignin, soluble phenolics) (Table 4; Fig. 3). This is shown graphically in Fig. 4, where the differences in chemical composition between the deciduous and

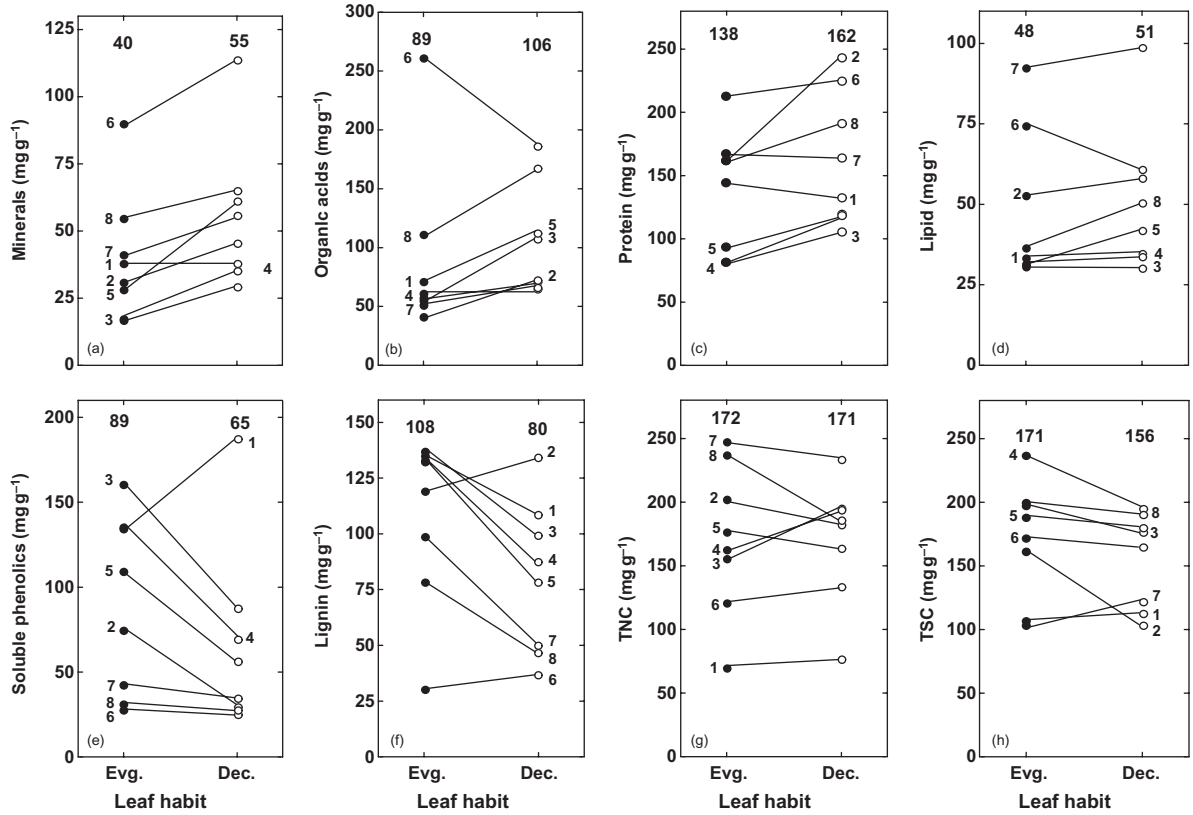

Figure 2. Leaf chemical composition for evergreen (Evg.) and deciduous (Dec.) species of the eight pairs. (a) Minerals; (b) organic acids; (c) protein; (d) lipid; (e) soluble phenolics; (f) lignin; (g) total non-structural carbohydrates (TNC); and (h) total structural carbohydrates (TSC). Lines connect the points for each evergreen–deciduous pair (see Table 1 for codes of the pairs). Numbers in bold in each panel indicate mean values for evergreen and deciduous species. Shaded and unshaded circles represent evergreen and deciduous species, respectively.

Table 4. Correlations between mean values of leaf chemical compounds, construction costs (CC), relative growth rate (RGR) and specific leaf area (SLA) in the 16 species studied

	Organic acids	Protein	Lipid	Soluble phenolics	Lignin	TSC	TNC	Carbon	C/N ratio	CC	RGR	SLA
Minerals	0.82***	0.73***	0.46[a]	−0.66**	−0.85***	−0.11[ns]	−0.14[ns]	−0.85***	−0.80***	−0.61*	0.88***	0.89***
Organic acids		0.52*	0.21[ns]	−0.52*	−0.79***	0.21[ns]	−0.21[ns]	−0.97***	−0.59*	−0.88***	0.60*	0.77***
Protein			0.60*	−0.71**	−0.52*	−0.48[a]	0.02[ns]	−0.48[a]	−0.95***	−0.10[ns]	0.83***	0.83***
Lipid				−0.62*	−0.53*	−0.51*	0.38[ns]	−0.15[ns]	−0.59*	0.11[ns]	0.56*	0.43[a]
Soluble phenolics					0.68*	0.06[ns]	−0.55*	0.58*	0.69**	0.45[ns]	−0.75***	−0.84***
Lignin						−0.07[ns]	−0.13[ns]	0.84***	0.67**	0.70**	−0.71**	−0.75***
TSC							0.15[ns]	−0.31[ns]	0.46[a]	−0.58*	−0.33[ns]	−0.17[ns]
TNC								0.11[ns]	−0.03[ns]	0.13[ns]	−0.07[ns]	−0.13[ns]
Carbon									0.57*	0.92***	−0.60*	−0.76***
C/N ratio										0.23[ns]	−0.84***	−0.81***
CC											−0.29[ns]	−0.49[a]
RGR												0.89***

Asterisks show the significance level of correlations.
$^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.
[a]$0.05 < P < 0.10$; ns, non-significant.
TSC, total structural carbohydrates; TNC, total non-structural carbohydrates; C/N, carbon/nitrogen.

Figure 3. Principal component analysis (PCA) of different chemical compounds, leaf construction costs (Con. costs), carbon/nitrogen (C/N) ratio and relative growth rate (RGR). The two factors together explain 79% of the variance.
TNC, total non-structural carbohydrates; TSC, total structural carbohydrates.

evergreen species of each PIC are plotted against the impact that this variation has on the difference in CC. Five compounds are mainly responsible for the small differences in CC between deciduous and evergreen species. Deciduous leaves have higher levels of protein (which increase CC) and higher concentrations of organic acids and minerals (which decrease CC). Moreover, deciduous leaves have lower concentrations of lignin and soluble phenolics (which decrease CC). Other compounds, such as TSC or TNC, may differ largely in concentration between PIC pairs, but only exert a small impact on CC, because the specific glucose costs of these compounds differ little from the overall CC of the plant material. Therefore, it must be pointed out that high variability in chemical composition between different pairs exists (Fig. 4). For example, the

Figure 4. Differences in leaf construction costs (CC) between deciduous and evergreen species of each pair (PIC) plotted as a function of the differences in chemical composition between the species of both functional groups.

differences in protein concentration between the deciduous and evergreen species of all pairs range from –12 to 82 mg g^{-1} (Fig. 4).

Relationship of chemical composition and CC with RGR

Fast-growing woody species had higher concentrations of minerals, organic acids, protein and lipids than slow-growing species (Fig. 5a–d; Table 4), but had lower concentrations of soluble phenolics, lignin and carbon (Fig. 5e & f; Table 4), and a lower C/N ratio (Table 4). No correlation with RGR was found for TNC or TSC (Fig. 5g & h). However, this pattern is not universally valid for all PICs. For example, in the relationship of RGR and soluble phenolics, the species of pairs 1, 5, 6, 7 and 8 do not follow the general pattern (i.e. a decrease in RGR is not associated with a decrease in soluble phenolics). The same is true for some of the other relationships (Fig. 5).

There was no correlation between leaf CC and RGR ($r = -0.29$, $P > 0.2$; Fig. 6a), but there was a negative correlation of RGR and CC of stems and roots ($r = -0.63$, $P < 0.01$ and $r = -0.54$; $P < 0.05$, respectively, Fig. 6b & c). Because most of the plant biomass was invested in leaves ($46 \pm 11\%$, average over all species), the correlation between RGR and CC_w was not significant either ($r = -0.41$; $P > 0.1$; Fig. 6d). Similar to the relationships of RGR with chemical composition, the pattern for all the PICs does not follow a general trend, indicating that the relationships between RGR and CC are not general for the evergreen and deciduous characters.

DISCUSSION

Construction costs

Differences in leaf CC of evergreen and deciduous species were investigated before in several ecosystems. About 20%

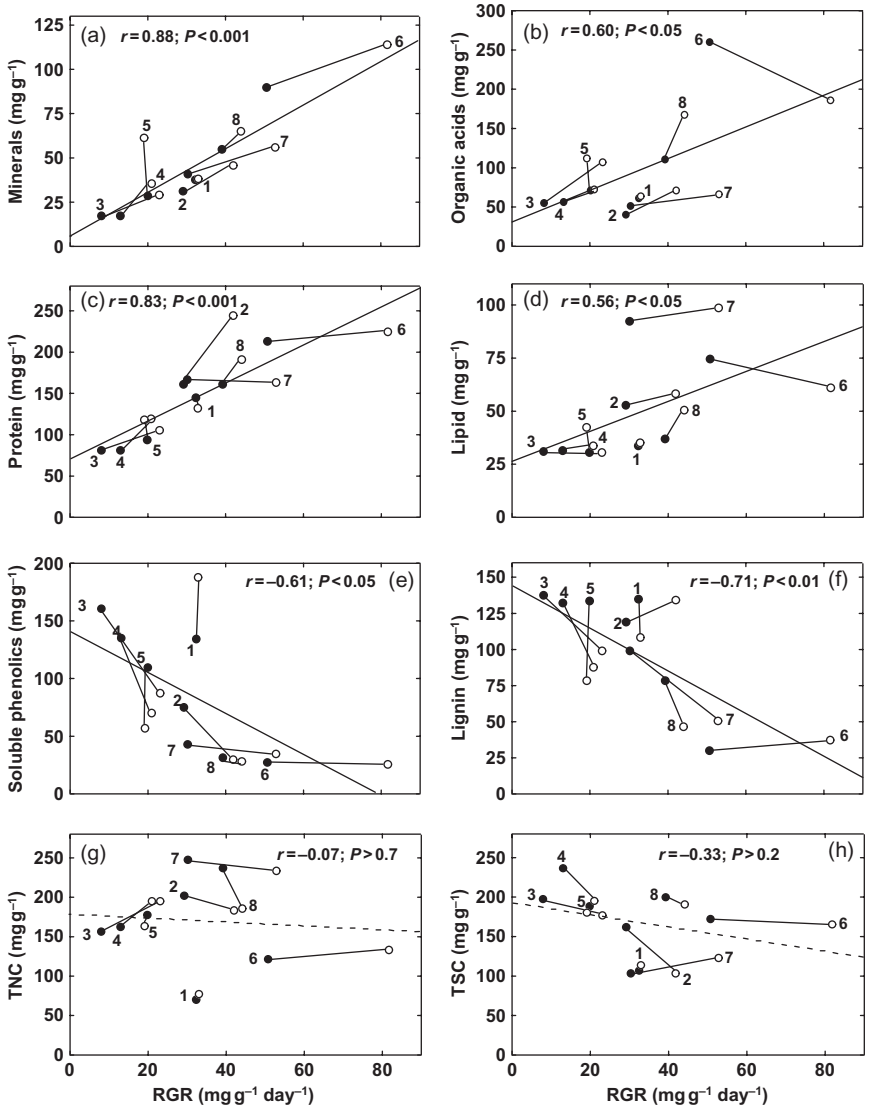

Figure 5. Concentrations of eight different classes of compounds in leaves of 16 woody species as a function of the relative growth rate (RGR) of these species. (a) Minerals; (b) organic acids; (c) protein; (d) lipid; (e) soluble phenolics; (f) lignin; (g) total non-structural carbohydrates (TNC); and (h) total structural carbohydrates (TSC). Solid thick lines indicate a significant correlation across species ($P < 0.05$), broken lines indicate a non-significant correlation ($P > 0.05$). Thin lines connect the points for each evergreen–deciduous pair (see Table 1 for codes of the pairs). Shaded and unshaded circles represent evergreen and deciduous species, respectively.

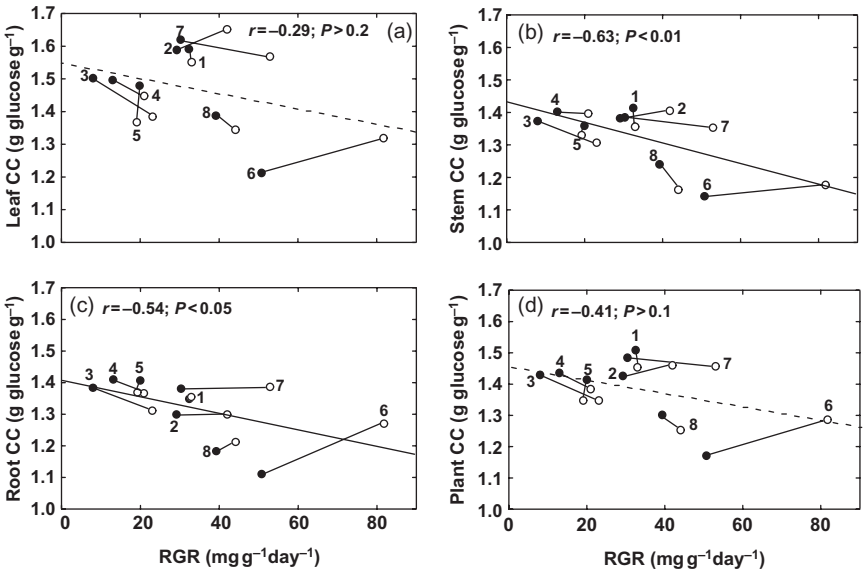

Figure 6. Construction costs (CC) of (a) leaf, (b) stem, (c) root and (d) whole plant of 16 woody species plotted as a function of the relative growth rate (RGR) of these species. Solid thick lines indicate a significant correlation across species ($P < 0.05$), broken lines indicate a non-significant correlation ($P > 0.05$). Thin lines connect the points for each evergreen–deciduous pair (see Table 1 for codes of the pairs). Shaded and unshaded circles represent evergreen and deciduous species, respectively.

higher CC values were found in evergreens by Miller & Stoner (1979), but these values were based on an incomplete analysis of the leaves. Merino (1987) found about 7% higher leaf CC of evergreen Mediterranean species than deciduous ones. Sobrado (1991) reported up to 100% higher values for evergreen species in the tropics. However, this was attributed to the fact that she expressed CC on an area basis. On a mass basis, no differences were observed. Slightly higher values (6% on average) in evergreens were observed by Villar & Merino (2001) for a range of 162 species from 14 different ecosystems. They also found that within each of the functional groups, there was a wide variation among species, values ranging from 1.2 to 1.9 g glucose g^{-1}, similarly as Merino (1987). Higher values in evergreens were also reported for Australian savannah species (Eamus & Prichard 1998). Navas *et al*. (2003), however, did not find a significant effect of leaf habit on CC for a range of Mediterranean species.

Most of these comparisons between deciduous and evergreens were carried out for plants growing in the field and therefore, different species may have grown under different environmental conditions. As pointed out in the Introduction section, CC is affected by different environmental factors (N and P soil availabilities, CO_2 and irradiance). Martinez *et al*. (2002), for example, found that the differences observed in root CC among shrubs, herbaceous and woody species were affected more by habitat differences than by intrinsic differences among the species. To exclude confounding effects of environmental conditions, we grew all our plants under identical, semi-controlled conditions. Our results showed no significant difference in leaf CC between the eight evergreen and eight deciduous species. Similarly, Chapin (1989) did not find a difference in the leaf CC of deciduous and evergreen species growing at the same location in the tundra.

With variation being large among species and rather small between functional groups, there is a second point of concern. It might well be that differences are attributed more to constraints in the phylogeny than to the functional differences imposed by natural selection. The aspect of phylogeny has hardly received any attention in this field of research. The present experiment was set up specifically to address this question by comparing the functional groups within family-related pairs. Indeed, we observed strong effects of family on leaf CC. Altogether, we conclude that a variation in CC exists between families of species, but that it does not play a role in differentiating deciduous and evergreen species. Similarly, Baruch & Goldstein (1999) found only a 3% difference in leaf CC between invasive and native species from the same families. This contrasts with other variables, such as SLA, which show strong contrasts between the two functional groups of species (Reich 1998; Antunez *et al*. 2001; Ruiz-Robleto & Villar 2005).

The same conclusion as for leaf CC holds for the CC of stems and roots. No systematic differences were found between the functional groups and large differences between families. Martinez *et al*. (2002) found higher root CC for evergreens than for deciduous, as a result of the higher investment in waxes and lower investment in cellulose by evergreens. Although all these species were from the *Quercus* genus, they were collected in different habitats with contrasting fertility, which may have affected the results, as was pointed by the authors. An alternative explanation that we cannot completely rule out is that there are strong species–environment interactions for CC, such as those observed by Niinemets (1999) or Poorter *et al*. (2006). This could imply that CC differences between deciduous and evergreen species do not show up under the glasshouse conditions of the present experiment, but become apparent under strongly limiting nutrient or water levels (cf. Martinez *et al*. 2002). This alternative hypothesis awaits further testing, although most environmental effects on CC up to now were found to be small (Poorter & De Jong 1999).

Comparison of CC of stems and roots with those of leaves, showed that leaves are more costly to build. A

similar conclusion was reached for 24 herbaceous species (Poorter & Bergkotte 1992) and for *Lycopersicon* plants (Gary *et al*. 1998). This may well be attributed to the higher carbon and protein concentrations of leaves as compared to stems and roots.

In the previous discussion, we have considered leaf, stem and root CC separately. However, leaves cannot function without stems and roots and vice versa, so insight at the whole-plant level can only be obtained if also the CC per unit total biomass are considered (Poorter 1994). CC_w values were slightly but non-significantly higher for evergreen species (Appendix II), whereas differences between families are dominant. Few data exist for whole plants. In line with the present results, Poorter & Bergkotte (1992) did not find differences in CC_w between slow- and fast-growing herbaceous species.

Chemical composition

The present values for the concentrations of the different chemical compounds were in the range of those for woody species observed in the literature compilation of Poorter & Villar (1997), with the exception of the organic acids, which showed very high values for the species of the *Ficus* genus. Surprisingly, the main factor causing differences in chemical composition was not the deciduous–evergreen character, but the phylogeny (family). This was contrary to the general consideration that there are clear differences between both functional groups in chemical composition (e.g. deciduous species are considered to have high concentrations of protein, minerals and organic acids, whereas evergreens were considered to have high lignin and phenol concentration) (Loveless 1962, Coley 1988; Reich, Walters & Ellsworth 1992; Villar, Held & Merino 1995; Cornelissen *et al*. 1997; Reich, Walters & Ellsworth 1997). However, our results do not support this idea and also in some other recent studies, a large overlap in different characteristics of evergreen and deciduous species was found (Wright *et al*. 2005).

Baruch & Goldstein (1999) found differences in N and P concentrations, photosynthetic rate and CC between invasive and native species, but these differences do not appear as clear when these two groups were compared within the same family, also stressing the importance of phylogeny. Similarly, Martinez *et al*. (2002) found large differences in chemical composition of roots between different families of shrubs (Labiatae versus Cistaceae). Although the importance of family on the chemical composition was recognized widely (Whittaker 1970; Font-Quer 1993), up to now, we are not aware of any study comparing the importance of phylogeny and functional group (deciduous versus evergreen) on chemical composition. In summary, we found no differences between deciduous and evergreens in chemical composition but large variation in chemical composition between genera.

Comparing the chemical composition between leaves, stems and roots we found that it was very well related. Poorter & Villar (1997) also found similar results in 24 herbaceous species. Also, in the medical plant literature (e.g. Font-Quer 1993), it is normally found that a chemical principle is present in all the plant organs (fruits, leaves, stems or roots).

The CC is a valuable parameter to know if one is interested in the C-budget of plants. However, it is also a 'black box', as the underlying reasons for values being different or similar across species or treatments do not become clear. This requires insight into the chemical composition of plants. It was found in different studies that the existence of correlations among chemical compounds are one of the reasons for CC values being constant (Chapin 1989; Poorter & Bergkotte 1992; Villar & Merino 2001; Martinez *et al*. 2002). Some of these correlations were either: (1) positive correlations between expensive and inexpensive fractions (for example: proteins with minerals in Poorter & Bergkotte 1992; Villar & Merino 2001 and in our study or soluble phenolics with minerals in Martinez *et al*. 2002); (2) negative correlations between expensive compounds (lignin and protein in Chapin 1989 and in our study or waxes and proteins in Martinez *et al*. 2002); or (3) negative correlations between inexpensive compounds (minerals and cellulose in Martinez *et al*. 2002). However, it may happen also a negative correlation between a very expensive compound and a very cheap one, which would enhance the differences and may be responsible of marked differences between functional groups. This occurs in the Martinez *et al*. (2002) study, where wax (expensive) was negatively correlated with cellulose (cheap) and being responsible for the differences between root CC of evergreen and deciduous oaks. In our study, soluble phenolics and minerals were negatively correlated, but their impact on CC was reduced as a result of the negative correlation of soluble phenolics with two other expensive constituents (protein and lipid).

Relationship of chemical composition and CC with RGR

Interspecific variation in chemical composition is related to the RGR. Results from this study with 16 woody species are similar to those of Poorter & Bergkotte (1992) with 24 herbaceous species. In both studies, RGR was positively correlated with minerals, organic acids and protein, whereas growth rate was negatively correlated with lignin and carbon, suggesting that the presently observed patterns are rather general. Some compounds (e.g. proteins) are related to the primary metabolism or growth of a plant, and generally a positive correlation is found between protein concentration and maximum photosynthetic rate (Reich *et al*. 1997; Wright *et al*. 2004), protein and respiration rate (Atkin, Botman & Lambers 1996; Reich *et al*. 1998) or protein with RGR (Poorter & Bergkotte 1992; Cornelissen *et al*. 1997).

Other chemical compounds, such as soluble phenolics, lignin and terpenes, were related to a defence role. Plant species with high concentrations of such compounds generally suffer less from consumption by herbivores and hence, have a higher chance of survival (Coley 1988; Granado &

Caballero 2001). Moreover, the chemical composition of a plant seems to have a high importance for decomposers as well because plant material with high protein concentration is preferentially selected by decomposers, similar to what is found for herbivores (Cornelissen *et al*. 1999; Gallardo & Merino 1999). Plant material with high concentration of tannins, phenols or terpenes, however, are less easily decomposed (Grime *et al*. 1996), limiting the energy flow to higher trophic levels and slowing down the nutrient cycle.

As a plant has a limited amount of energy, it has been hypothesized that there is a trade-off between growth and defence (Herms & Mattson 1992). The relationships between chemical compounds and RGR found in our study agree with this hypothesis. Species with high growth potential invest more energy in primary compounds (proteins) and less in secondary compounds with a possible defence role (such as phenols or lignin) and vice versa. Other studies have found similar results (Coley 1988; Poorter & Bergkotte 1992; Van Arendonk & Poorter 1994). However, not all studies support this view. For example, in a study of 14 species of the *Hakea* genus, Hanley & Lamont (2002) found a positive relationship between RGR and phenol concentration, which is contrary to the suggested trade-off of defence and growth. Similarly, Almeida-Cortez, Shipley & Arnason (1999) found a positive correlation between RGR and phenolic concentration in 31 herbaceous species from Asteraceae. They did not find any relationship between potential tissue toxicity and RGR either.

We found that the relationships between RGR and chemical concentration are not always clear for all the PICs (e.g. the negative correlation between RGR and soluble phenolics is not clear for most of the deciduous–evergreen pairs). This suggests that although the hypothesis is verified in general, it may not be true for specific groups with different phylogenetic and ecological constraints. Contrary to what was expected (see Introduction section), CC of leaves, stems, roots or whole plants showed weak or non-existing correlations with RGR, suggesting that data on CC were a poor predictor for potential growth. This seems at variance with Nagel *et al*. (2004), who found that a lower CC is associated with a higher biomass, RGR and seed production. In that study, the lower CC was not attributed to a decrease in N concentration, but probably to an accumulation of inexpensive carbohydrates. Therefore, the biomass is cheaper to build, but the potential to capture CO_2 by photosynthesis is not lower. However, the decrease in CC (about 11%), found in Nagel *et al*. (2004), did not fully explain the increase in RGR (about 41%). Nagel & Griffin (2001) also found that a lower CC might be advantageous as it is related with a high relative abundance. However, Poorter & de Jong (1999) did not find any relationship between CC and above-ground production in 15 habitats, although C/N ratio showed a negative relationship with above-ground production. This is in accordance with our results as C/N ratio is also negatively related to RGR and is one of the variables more highly correlated with RGR. Therefore, we stress the importance of the C/N ratio as a better predictor of growth and productivity than CC.

CONCLUSIONS

The variation in CC and chemical composition in leaf, stem and root are attributed mainly to phylogeny (family) and not to leaf habit (evergreen versus deciduous). Chemical composition of leaves, stems and roots are well correlated. Fast-growing species invest more in minerals, organic acids, proteins and lipid but less in lignin and soluble phenolics, which suggests a trade-off between growth and defence. The C/N ratio emerges as a better predictor of potential growth rate than CC.

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APPENDIX I

Mean values [± standard deviation (SD)] of leaf chemical composition, carbon/nitrogen (C/N) ratio and construction cost (CC) of the eight phylogenetic independent contrasts (PIC) between evergreens and deciduous species

Species	Leaf habit	PIC code	Minerals (mg g^{-1})	Organic acids (mg g^{-1})	Protein (mg g^{-1})	Lipid (mg g^{-1})	Soluble phenols (mg g^{-1})	Lignin (mg g^{-1})	TNC (mg g^{-1})	TSC (mg g^{-1})	C/N (g C/g N)	CC (g glucose g^{-1})
Pistacia terebinthus	D	1	38.1 ± 9.2	64.6 ± 16.3	132.1 ± 14.0	34.8 ± 7.7	187.3 ± 4.3	108.5 ± 10.3	76.6 ± 4.6	113.1 ± 12.7	22.0 ± 2.1	1.55 ± 0.01
Pistacia lentiscus	E		37.9 ± 3.1	61.8 ± 4.7	144.5 ± 7.5	33.4 ± 4.4	134.2 ± 10.7	134.7 ± 30.0	69.7 ± 4.8	106.8 ± 34.9	20.6 ± 1.0	1.59 ± 0.05
Gleditsia triacanthos	D	2	45.5 ± 10.2	71.3 ± 10.7	243.6 ± 17.2	58.2 ± 6.1	29.7 ± 0.3	134.1 ± 12.3	182.9 ± 15.5	103.1 ± 23.4	12.1 ± 0.9	1.65 ± 0.02
Ceratonia siliqua	E		31.1 ± 7. 9	41.1 ± 17.7	161.3 ± 11.5	52.9 ± 4.5	75.0 ± 7.0	118.9 ± 4.3	202.0 ± 16.6	161.5 ± 9.3	18.6 ± 1.5	1.59 ± 0.01
Castanea sativa	D	3	29.2 ± 6.2	108.0 ± 40.3	105.0 ± 15.8	30.2 ± 10.3	87.5 ± 25.8	99.1 ± 5.1	195.3 ± 23.2	176.8 ± 5.4	26.1 ± 4.3	1.38 ± 0.03
Quercus coccifera	E		17.0 ± 12.1	55.2 ± 23.8	81.3 ± 6.2	31.0 ± 2.7	160.3 ± 10.7	137.2 ± 4.7	156.2 ± 11.4	197.4 ± 12.4	36.0 ± 2.9	1.50 ± 0.01
Quercus pyrenaica	D	4	35.4 ± 4.5	72.8 ± 20.6	119.1 ± 3.6	33.6 ± 2.6	69.7 ± 12.2	87.3 ± 17.3	194.3 ± 30	195.2 ± 18.4	23.3 ± 0.4	1.45 ± 0.02
Quercus ilex ssp. *ballota*	E		17.3 ± 9.4	57.0 ± 20.0	81.1 ± 9.6	31.6 ± 5.6	135.3 ± 26.4	132.3 ± 11.1	162.2 ± 9.4	236.6 ± 21.9	35.5 ± 3.8	1.50 ± 0.02
Quercus robur	D	5	61.0 ± 23.8	112.6 ± 48.5	117.7 ± 6.7	42.1 ± 3.5	56.6 ± 1.1	78.5 ± 3.1	163.7 ± 11.0	180.0 ± 8.4	22.8 ± 1.4	1.37 ± 0.03
Quercus suber	E		28.4 ± 9.3	71.4 ± 16.8	93.4 ± 1.1	30.4 ± 3.6	109.4 ± 14.0	133.3 ± 4.7	177.1 ± 5.8	188 ± 16.4	30.2 ± 0.3	1.48 ± 0.02
Ficus carica	D	6	113.8 ± 19.3	186.6 ± 31.8	224.9 ± 30.8	61.0 ± 20.2	25.5 ± 0.3	37.2 ± 7.3	133.5 ± 29.1	165.2 ± 3.9	9.9 ± 1.6	1.32 ± 0.04
Ficus retusa	E		89.7 ± na	260.8 ± na	212.6 ± na	74.4 ± na	27.53 ± na	30.2 ± na	120.9 ± na	172.3 ± na	9.5 ± na	1.21 ± na
Fraxinus angustifolia	D	7	55.6 ± 11.6	66.6 ± 24.8	163.6 ± 17.5	98.6 ± 2.0	34.36 ± 0.63	50.3 ± 8.2	233.5 ± 16.0	122.5 ± 15.7	17.8 ± 1.6	1.57 ± 0.06
Olea europaea ssp. *sylvestris*	E		41.1 ± 5.9	51.8 ± 20.1	166.8 ± 1.2	92.4 ± 23.2	42.4 ± 2.5	98.8 ± 27.2	247.1 ± 65.1	103.0 ± 20.3	17.9 ± 0.3	1.62 ± 0.02
Sterculia platanifolia	D	8	64.8 ± 5.7	167.3 ± 29.2	191.2 ± 17.4	50.4 ± 6.4	27.9 ± 0.3	46.5 ± 0.4	185.6 ± 48.7	190.3 ± 7.8	13.6 ± 1.4	1.34 ± 0.02
Sterculia diversifolia	E		54.8 ± 6.1	111.3 ± 32.7	161.3 ± 8.4	36.7 ± 4.5	30.9 ± 0.6	78.5 ± 16.1	236.7 ± 51.4	199.6 ± 32.6	16.5 ± 1.3	1.39 ± 0.03
Mean ± SD			47.5 ± 25.8	97.5 ± 60.0	150.0 ± 50.2	49.5 ± 22.3	77.1 ± 53.1	94.1 ± 37.4	171.1 ± 51.6	163.2 ± 41.1	20.8 ± 8.1	1.47 ± 0.12
Coefficient of variation (%)			54	62	33	45	69	40	30	25	39	8

Values are averages of three-bulk-sample determinations. At the bottom of the table, mean ± SD across species as well as the coefficient of variation are given.
TNC, total non-structural carbohydrates; TSC, total structural carbohydrates; na, not available.

APPENDIX II

Mean values [± standard deviation (SD)] of chemical composition, carbon/nitrogen (C/N) ratio and construction cost (CC) of stems and roots and whole plant CC of the eight phylogenetic independent contrasts (PIC) between evergreens and deciduous species

			Stem					Root					Whole plant
Species	Leaf habit	PIC code	Minerals (mg g^{-1})	Organic acids (mg g^{-1})	Protein (mg g^{-1})	C/N (g C/g N)	CC (g glucose g^{-1})	Minerals (mg g^{-1})	Organic acids (mg g^{-1})	Protein (mg g^{-1})	C/N (g C/g N)	CC (g glucose g^{-1})	CC (g glucose g^{-1})
Pistacia terebinthus	D	1	33.8 ± 7.7	71.0 ± 12.0	64.1 ± 0.6	42.9 ± 0.9	1.36 ± 0.01	41.7 ± 13.4	56.8 ± 10.5	79.8 ± 25.7	36.5 ± 13.3	1.35 ± 0.06	1.45 ± 0.013
Pistacia lentiscus	E		35.4 ± 3.4	74.7 ± 11.9	80.2 ± 2.9	34.4 ± 1.9	1.41 ± 0.03	40.5 ± 2.8	44.8 ± 5.1	88.1 ± 21.4	30.4 ± 7.4	1.35 ± 0.04	1.50 ± 0.035
Gleditsia triacanthos	D	2	25.1 ± 10.4	35.4 ± 24.2	94.1 ± 12.3	30.5 ± 3.8	1.40 ± 0.01	98.2 ± 78.0	70.1 ± 46.7	117.7 ± 8.4	24.3 ± 11.0	1.30 ± 0.08	1.46 ± 0.021
Ceratonia siliqua	E		32.7 ± 9.7	54.1 ± 13.8	83.2 ± 18.7	34.5 ± 8.0	1.38 ± 0.02	67.7 ± 33.7	52.6 ± 11.6	119.2 ± 6.4	21.9 ± 6.9	1.30 ± 0.02	1.42 ± 0.005
Castanea sativa	D	3	21.8 ± 4.6	101.0 ± 5.4	79.6 ± 17.2	34.7 ± 8.0	1.31 ± 0.01	51.2 ± 48.8	65.6 ± 23.3	79.6 ± 42.7	40.3 ± 27.9	1.31 ± 0.01	1.34 ± 0.005
Quercus coccifera	E		26.4 ± 10.3	78.1 ± 13.8	53.9 ± 10.6	52.0 ± 9.1	1.37 ± 0.02	27.2 ± 7.2	41.2 ± 3.2	68.6 ± 8.6	41 ± 5.2	1.38 ± 0.01	1.42 ± 0.012
Quercus pyrenaica	D	4	36.5 ± 8.5	56.9 ± 30.6	81.5 ± 17.5	35.2 ± 8.4	1.40 ± 0.03	29.2 ± 2.6	58.3 ± 13.6	113.9 ± 2.2	24.1 ± 2.3	1.37 ± 0.01	1.38 ± 0.003
Quercus ilex ssp. *ballota*	E		27.7 ± 6.1	66.2 ± 27.0	55.3 ± 5.1	51.3 ± 3.8	1.40 ± 0.01	36.6 ± 12.9	41.7 ± 14.1	68.2 ± 13.1	41.8 ± 8.5	1.41 ± 0.01	1.43 ± 0.012
Quercus robur	D	5	32.7 ± 16.2	67.1 ± 24.2	63.1 ± 7.7	44.4 ± 6.0	1.33 ± 0.03	25.2 ± 12.7	46.3 ± 5.5	71.6 ± 21.0	40.6 ± 13.5	1.37 ± 0.01	1.34 ± 0.018
Quercus suber	E		23.9 ± 0.9	101.9 ± 1.5	57.9 ± 4.8	48.7 ± 3.8	1.36 ± 0.01	31.5 ± 5.3	51.3 ± 8.8	102.5 ± 8.5	27.8 ± 5.1	1.41 ± 0.02	1.41 ± 0.001
Ficus carica	D	6	86.7 ± 19.7	87.8 ± 3.7	105.9 ± 2.2	19.9 ± 7.1	1.18 ± 0.08	65.5 ± 7.6	76.6 ± 16.3	104.2 ± 5.5	23.0 ± 5.1	1.27 ± 0.02	1.28 ± 0.020
Ficus retusa	E		76.4 ± na	178.0 ± na	137.7 ± na	13.7 ± na	1.14 ± na	76.5 ± na	69.1 ± na	92.7 ± na	20.8 ± na	1.11 ± na	1.17 ± na
Fraxinus angustifolia	D	7	20.6 ± 4.5	47.6 ± 5.5	60.6 ± 13.1	48.1 ± 12.3	1.35 ± 0.02	41.2 ± 18.4	54.4 ± 15.7	87.5 ± 26.4	34.3 ± 13.8	1.39 ± 0.03	1.45 ± 0.035
Olea europaea ssp. *sylvestris*	E		29.7 ± 3.6	61.8 ± 5.4	77.5 ± 6.5	36.2 ± 2.4	1.39 ± 0.01	34.7 ± 12.6	49.0 ± 4.4	89.5 ± 21.5	32.0 ± 7.0	1.38 ± 0.02	1.48 ± 0.004
Sterculia platanifolia	D	8	50.7 ± 5.8	114.9 ± 25.2	64.7 ± 14.0	36.1 ± 7.7	1.16 ± 0.03	67.8 ± 25.2	85.1 ± 10.2	94.2 ± 30.9	26.1 ± 11.6	1.21 ± 0.05	1.25 ± 0.013
Sterculia diversifolia	E		63.83 ± 8.8	80.2 ± 11.3	58.9 ± 9.0	39.9 ± 8.4	1.24 ± 0.01	100.6 ± 7.3	94.8 ± 18.9	82.6 ± 29.5	23.2 ± 10.2	1.18 ± 0.03	1.30 ± 0.018
Mean ± SD			39.0 ± 20.0	79.8 ± 33.5	76.2 ± 22.1	37.7 ± 10.6	1.32 ± 0.09	52.2 ± 24.4	59.9 ± 15.8	91.3 ± 16.5	30.5 ± 7.6	1.32 ± 0.09	1.38 ± 0.09
Coefficient of variation (%)			51	42	29	28	7	47	26	18	25	7	7

Values are averages of three-bulk-sample determinations. At the bottom of the table, mean ± SD across species as well as the coefficient of variation are given.
na, not available.

