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The effect of elevated CO_2 on the chemical composition and construction costs of leaves of 27 C_3 species

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ABSTRACT

We determined the proximate chemical composition as well as the construction costs of leaves of 27 species, grown at ambient and at a twice-ambient partial pressure of atmospheric CO_2. These species comprised wild and agricultural herbaceous plants as well as tree seedlings. Both average responses across species and the range in response were considered. Expressed on a total dry weight basis, the main change in chemical composition due to CO_2 was the accumulation of total non-structural carbohydrates (TNC). To a lesser extent, decreases were found for organic N compounds and minerals. Hardly any change was observed for total structural carbohydrates (cellulose plus hemicellulose), lignin and lipids. When expressed on a TNC-free basis, decreases in organic N compounds and minerals were still present. On this basis, there was also an increase in the concentration of soluble phenolics.

In terms of glucose required for biosynthesis, the increase in costs for one chemical compound - TNC - was balanced by a decrease in the costs for organic N compounds. Therefore, the construction costs, the total amount of glucose required to produce 1 g of leaf, were rather similar for the two CO_2 treatments; on average a small decrease of 3% was found. This decrease was attributable to a decrease of up to 30% in the growth respiration coefficient, the total CO_2 respired [mainly for NAD(P)H and ATP] in the process of constructing 1 g of biomass. The main reasons for this reduction were the decrease in organic N compounds and the increase in TNC.

Key-words: carbon dioxide; chemical composition; C:N ratio; construction costs; growth respiration; lignin; minerals; organic acids; protein; TNC.

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INTRODUCTION

The stimulating effect of a high atmospheric CO_2 partial pressure on the growth of C_3 plants is not well understood. On average, a doubling in ambient pCO_2 increases plant weight by *c.* 40% (Poorter *et al.* 1996). Such stimulation is modest compared to what might be expected on the basis of short-term increases in carbon fixation at high pCO_2. A feedback mechanism that down-regulates photosynthesis is one possible explanation for this phenomenon (cf. Sage 1994). However, other aspects of the metabolism of the plant may also change. Both increases and decreases in respiration have been found (Poorter *et al.* 1992; Amthor 1996), and in some cases a shift in biomass allocation from leaves to roots occurs (Stulen & Den Hertog 1993). In addition to these factors, altered growth due to high CO_2 may be caused by a change in the chemical composition of the plants. It is this possibility that is addressed in this paper.

The wide variety of quantitatively important chemical compounds can be roughly grouped into eight different classes: lipids, soluble phenolics, proteins, lignins, total structural carbohydrates (TSC; cellulose, hemicellulose and pectin), total non-structural carbohydrates (TNC; starch, fructan, sucrose, etc.), organic acids and minerals. For each of the organic constituents of a plant, a certain amount of carbon is required for C skeletons. Reduced C is also necessary to generate the NAD(P)H and ATP which is converted during the synthesis of the organic compounds. Given the biosynthetic pathways, and assuming glucose as a starting point, the total costs for producing 1 g of each of these constituents can be calculated (Penning de Vries, Brunsting & Van Laar 1974). Some groups of chemical constituents are relatively expensive to produce (2.1-3.0 g glucose g^{-1} for lipids, soluble phenolics, proteins and lignins), whereas others (such as TNC and organic acids) are formed with relatively small amounts of glucose (0.9-1.2 g glucose g^{-1}). A separate group is that of the minerals, which do not require glucose, apart from the energy costs for uptake and transport. The consequence of the

rather large variation in synthesis costs between various plant compounds is that the construction costs, the total amount of glucose required to produce 1 g of plant biomass, may alter if chemical composition is affected by growth conditions. Thus, to obtain a more general understanding of the growth response of plants to elevated pCO_2, it is necessary to know what changes in chemical composition will occur. Although some changes are well documented, especially the increase in TNC (e.g. Wong 1990; Körner & Miglietta 1994), a complete picture of the effects of high pCO_2 on the proximate chemical composition of the plant is not yet available.

Knowledge of the changes in composition will also provide more insight into some other aspects of plant performance. First, if the proximate chemical composition of high-CO_2 plants is known, it can be calculated how and why growth respiration (the amount of CO_2 produced or O_2 consumed per gram of new biomass produced) is affected (Penning de Vries *et al.* 1974). Growth respiration, together with respiration for maintenance and nutrient uptake, forms one of the major components of total plant respiration. Both increases and decreases in the rate of respiration are observed in response to high CO_2. As these changes are not fully understood, it is of interest to calculate how growth respiration is affected. Secondly, chemical composition may also affect the carbon budget of the plant indirectly. If high-CO_2 grown plants invest relatively more in cell walls and/or secondary compounds, this may increase their leaf longevity, but slow down their growth, whereas an additional investment in proteins (for example in photosynthetic machinery) may accelerate growth (Poorter & Bergkotte 1992).

In this paper, we characterize CO_2-induced changes in chemical composition and construction costs in the leaves of C_3 plants. As we are interested in both the average response and the ranges which are to be expected, we investigated the leaves of 27 species, including crop species and fast- and slow-growing wild herbaceous plants, as well as tree seedlings. Material was collected from different laboratories, with all plants grown at a relatively high level of nutrient and light supply, and at similar CO_2 partial pressures. However, as growth conditions were not exactly the same, a direct comparison between species is, strictly, precluded. On the other hand, this procedure enables a more general estimate of the range of responses which are to be expected for well-nourished plants grown at elevated pCO_2.

MATERIALS AND METHODS

Growth of the plants

The plant species used in this experiment are listed in Table 1. All species were grown at an elevated pCO_2 of between 68 and 72 Pa, and a control level of between 34 and 36 Pa, for a period of at least 20 d. Most species were grown in pots with soil, but a number of wild species were grown hydroponically. A summary of growth conditions, as well as the developmental stage of plants at harvest, is given in Table 1.

Chemical analyses

For each species, all leaves of different individuals were harvested at the same time and bulked into two independent groups. After either oven- or freeze-drying, the samples were ground to pass through a 0.08 mm sieve and redried.

Total C and N concentrations were determined with an elemental analyser (Carlo Erba, Milano, Italy), and the NO_3^- concentration in water extracts following Cataldo *et al.* (1975). Plant material was combusted in a muffle furnace at 550 °C for 6 h, and the remaining ash weighed. Subsequently, ash alkalinity was determined acidimetrically (Jungk 1968). Another series of analyses was carried out on separate subsamples. First, plant material was extracted with chloroform, methanol and water in a ratio of 2:2:1 (v/v) (Bligh & Dyer 1959). Lipids were determined gravimetrically on the residue left after drying of the chloroform phase over N_2. The soluble phenol content was analysed colorimetrically in the methanol-water phase with Folin-Ciocalteus reagent, using p-coumaric acid as a standard (Singleton 1988). Soluble sugars were also measured in this phase using the anthrone reagent (Fales 1951). The residue after extraction with chloroform, methanol and water was boiled for 2.5 h in 3% (v/v) HC1. Insoluble sugars were determined in the supernatant following Fales (1951). The remaining pellet was considered to be crude cell wall material and was weighed after drying. Thereafter, it was analysed for its C and N concentration as above.

Calculations

The weight of the organic N compounds was calculated by subtracting NO_3^--N from total N, and multiplying this value by 6.25. For ease of reference we will use the term 'protein' for this fraction throughout this paper. During the combustion process, NO_3^- and organic acids disappear, leaving an oxide, which reacts with CO_2 after cooling to form carbonate. Therefore, the total mineral content was calculated as the sum of ash and NO_3^- content, subtracting ash alkalinity (in milliequivalent g^{-1} DW) multiplied by the weight of 1 eq. of carbonate (30 g), to correct for the CO_3^{2-} formed. The organic acid concentration was determined by subtracting the NO_3^- concentration (in meq g^{-1}) from total ash alkalinity (in meq g^{-1}), and multiplying by 62.1 g eq^{-1}, assuming that the average weight of the organic acids per equivalent was similar to that determined previously for two species by gas chromatography (Poorter & Bergkotte 1992). The fraction that remained after the Bligh & Dyer extraction and the 3% HC1 treatment was considered to be composed of denatured cytoplasmic protein, cell wall protein, total structural carbohydrates (TSC) and lignin. In previous experiments we determined lignin

Table 1. Species used for the analyses, experimental conditions during growth, and some other characteristics of the plants harvested. Plants were grown in a glasshouse (Gh), growth chamber (GC) or in an open-top chamber (OTC). Maximum PPFD during growth is given, as well as the pot size. Those species grown in hydroponics are indicated by 'hy'. The duration of the experiment was such that in all cases > 95% of the biomass of the high-CO_2 plants had developed under high-CO_2 conditions. The number of plants is the total number of plants per treatment on which the analyses are based. In the last column it is indicated whether species show a symplastic (s) or an apoplastic (a) type of phloem loading. Species without an entry in this column either could not be classified or show an intermediate or mixed type of loading

Species name	Grown in:	Max. PPFD ($\mu mol\ m^{-2}\ s^{-1}$)	Pot size (L)	Length of experiment (day)	Developmental stage	No. of plants harvested	Type of loading
Crop species							
Brassica pekinensis L.	Gh	>1500	5.0	28	ve	16	a
Cucumis sativus L.	Gh	>1500	5.0	28	fr	16	s
Glycine max (L.) Merr.	Gh	>1600	3.5	50	fl	16	a
Gossypium hirsutum L.	GC	1000	0.4	28	fr	12	
Lolium perenne L.	GC	500	hy	21-36	ve	24	
Medicago sativa L.	GC	600	2.0	>30	ve	16	
Pisum sativum L.	GC	600	2.0	>30	fl	16	
Solanum tuberosum L.	Gh	>1200	2.0	45	fr	12	a
Triticum aestivum L.	Gh	>1200	5.0	45	fl	96	
Wild herbaceous species							
Abutilon theophrasti Medic.	Gh	1000	3.5	35	fl	12	s
Agrostis capillaris L.	OTC	>1100	0.8	105	ve	10	
Bromus erectus Huds.	GC	500	hy	26-42	ve	24	
Bromus hordaceus L.	GC	500	hy	23-38	ve	24	
Bromus madritensis L.	GC	500	hy	25-38	ve	24	
Bromus tectorum L.	GC	500	hy	26-40	ve	24	
Eichhornia crassipes (Mart.) Solms	GC	850	hy	16	ve	24	
Festuca vivipara L.	OTC	>1100	0.8	105	ve	16	
Trifolium subterraneum L.	GC	600	2.0	>30	ve	16	a
Plantago major L.	GC	550	hy	21	ve	16	a
Urtica dioica L.	GC	550	hy	21	ve	32	
Woody species							
Acacia auriculiformis Cunn. ex Benth.	Gh	>1500	5.0	60-70	ve	16	a
Acacia melanoxylon R. Br.	Gh	>1500	5.0	60-70	ve	16	a
Eucalyptus camaldulensis Dehnh.	Gh	>1500	5.0	60-70	ve	16	s
Eucalyptus cypellocarpa L. Johnson	Gh	>1500	5.0	60-70	ve	16	s
Eucalyptus pauciflora Sieb. ex Spreng	Gh	>1500	5.0	60-70	ve	16	s
Eucalyptus pulverulenta Sims	Gh	>1500	5.0	60-70	ve	16	s
Vitis vinifera L.	GC	600	2.0	>30	ve	16	s

following Morrison (1972). Like other methods for lignin determination, it is a difficult assay and rather indirect (cf. Boudet, Lapierre & Grima-Pettenati 1995). In principle it should be calibrated for each species. However, the problem is that pure lignin cannot be easily obtained and therefore this calibration is problematic. We chose to estimate lignin in an indirect way, based on the C and N concentration determined in the crude cell wall extract. Assuming the protein in the 'crude cell wall' pellet to have an average C and N concentration of 530 and 160 mg g^{-1}, respectively, we correct for the protein in the pellet remaining after the extractions. What is left is supposed to be a mixture of TSC and lignin. Lignin has a much higher weight fraction of C (*c.* 640 mg g^{-1}) than TSC (*c.* 440 mg g^{-1}), and therefore the proportion of lignin in this residue can be calculated. Using this method, we found lignin concentrations of 35 mg g^{-1} *in a Lolium perenne* vegetation, 106 mg g^{-1} in a mixture of *Erica tetralix* and *Molinia caerulea* (H. Poorter and R. de Jong, unpublished results), and 217 mg g^{-1} in a mixed *Eucalyptus/Pinus* wood powder sample. The last sample was previously found to have 239 mg g^{-1} lignin when analysed with the acetyl bromide method (Iiyama & Wallis 1990) after calibration against 100% alkali-extracted lignin from steam-exploded bagasse (R.M. Gifford, personal communication). For a more detailed description, see Poorter & Villar (1997).

The concentrations of the various compounds could be used to determine the construction costs of the plant material. However, as the sum of the compounds analysed never adds up fully to 100%, we preferred to estimate the construction costs indirectly, using the Vertregt & Penning de Vries (1987) method, slightly modified by Poorter (1994). In this approach, construction costs are derived from the C concentration of the plant material, as well as the mineral and protein concentrations. Included in this way are the costs of reducing nitrate to ammonium.

Growth respiration, in terms of CO_2 produced and O_2 consumed per gram of biomass synthesized, was calculated according to table 6 of Penning de Vries *et al.* (1974). In these calculations we assumed an average ADP:O ratio of 2.37 as derived from the compilation of a number of literature sources by Van der Werf *et al.* (1994).

Statistical analysis

As not all species were grown under similar conditions we calculated both the absolute and relative changes due to growth at high pCO_2 for each of the species and all of the eight compounds. The average difference over all species was calculated and confidence intervals determined to test whether the H_0 hypothesis of no change could be dismissed. Effects of pCO_2 on chemical composition were also analysed by an ANOVA, with species and pCO_2 as independent variables, to calculate what fraction of the total variation in the data was due to species, to pCO_2 and to the interaction of these two main effects. Although the possibility cannot be excluded that differences between species are in part attributable to variation in growth conditions, this allows an impression to be obtained of the importance of the CO_2 effect on chemical composition, relative to the effect of species.

To characterize the observed distribution of changes, percentiles were calculated and presented in box plots. The *x*th percentile indicates that *x%* of the observations are below that value, and 100 -*x%* above. The lower wide part of the boxes indicates the 25th percentile, and the higher the 75th percentile. The 'error bars' indicate the 10th and the 90th percentiles. The horizontal line in the middle of the box is the median value. Concentrations were arc-sine transformed, prior to the statistical analysis, to correct for the non-normal distribution of proportions. For a similar reason, C:N ratios were ln-transformed.

RESULTS

The concentrations of all of the investigated compounds in (generally mature) leaves of each species grown at either 35 or 70 Pa CO_2 are presented in Appendix 1. A summary is given in Table 2, with average values of the different compounds across all species, as well as the absolute changes due to growth in elevated pCO_2. Concentrations of all compounds, except soluble phenolics and lignin, were significantly affected by high pCO_2. However, for most of these constituents, the changes due to CO_2 were small, and explained less than 5% of the total variation, with differences between species (or experiments) being of overriding importance (ANOVA results; data not shown). There were two constituents on which CO_2 had a major effect. The most important effect was the increase in the concentration of total non-structural carbohydrates. Somewhat less important was the decrease in protein. The only other class of compounds for which the average (negative) change was larger than 10 mg g^{-1} was the minerals.

Table 2. Effect of a high CO2 partial pressure on the concentration of a number of compounds (in mg g^{-1} dry weight), as well as on the C:N ratio and the construction costs of leaves (in g glucose g^{-1} dry weight). Average values are given for 35 and 70 Pa CO_2-grown plants across the 27 species, as well as the significance of the absolute change in these parameters due to elevated CO2. ns, not significant; $*P < 0.05$; $**P < 0.001$, $***P<0.001$

Dependent	Average value		Absolute change
	35Pa CO_2	70Pa CO_2	
TNC	137	211	+74 ***
Sol. Phenolics	23	25	+2 ns
Lignin	40	40	0 ns
Lipids	57	53	-4 *
Org. acids	74	68	-7 *
TSC	142	132	- 10 ***
Minerals	91	75	- 16 ***
Protein	270	219	-49 ***
Insol. sugars	104	161	57 ***
Sol. sugars	31	46	15 ***
Total C	432	434	2 ns
NO_3	18	14	-4 **
Total N	48	38	-10 ***
C/N	9.2	11.8	_ ***
Construction costs	1.49	1.44	-0.05 ***

The above analysis is based on absolute values (mg compound per gram of dry weight). We also calculated, for all species, the concentration at high pCO_2 relative to that of control plants. These relative changes are not given for each species, but rather are presented as box plots (Figs 1 & 2). Box plots characterize a distribution of observed values as explained in 'Materials and methods'. It must be taken into account, in analysing differences in the concentrations of a compound, that concentrations may vary not only as a result of a change in the synthesis of that compound *per se,* but also as a result of a shift in the accumulation of any of the other constituents of the leaves. As the largest change for high-CO_2 grown plants is the increase in the concentration of total non-structural carbohydrates, we checked whether changes in other compounds were independent of the increase in TNC. To this end, concentrations of each constituent were calculated twice, first relative to the total dry weight (Fig. la), and secondly on a TNC-free basis (Fig. 1b).

When expressed on a relative basis, the major effect of CO_2 was again on the accumulation of TNC (Fig. la). There was also a significant increase in the concentration of soluble phenolics, as well as a decrease in the concentration of protein and minerals. The increase in soluble phenolics becomes more evident when considered on a TNC-free basis (Fig. 1b). The decreases in organic N and minerals are less pronounced, but remain significant. There was very little effect of the treatment on lignin, lipids or organic acids.

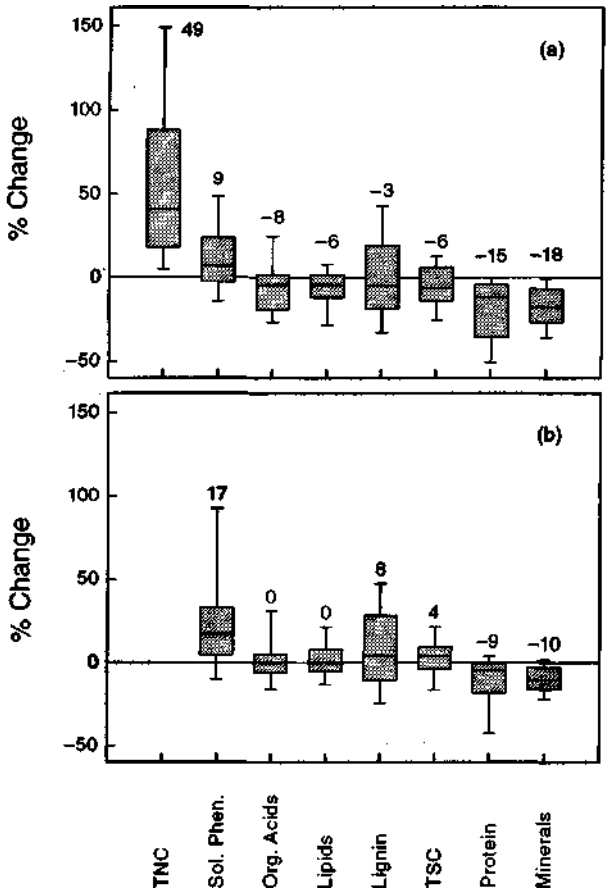

Figure 1. Proportional changes in leaf chemical composition of 70 Pa CO_2-grown plants relative to values for plants grown at 35 Pa CO_2. (a) Values on a total dry weight basis; (b) values on a TNC-free basis.

On average, there was no effect of a high pCO_2 on total C concentration (Fig. 2a). However, with the exception of three species, there was a clear negative relationship, with low-C plants increasing in C concentration, and high-C plants decreasing (Fig. 3a, $P < 0.001$). The concentration of total N decreased by a mean value of 18%. There was a difference in N decrease (on a TNC-free basis) between species grown in pots and those in hydroponics, in that pot-grown species decreased relatively more in N ($P < 0.001$; Fig. 3b). Largely as a consequence of the decrease in N, the C:N ratio increased by values of up to 80% (Fig. 2).

The coefficient of growth respiration, the amount of glucose respired or the quantity of CO_2 produced in the process of constructing 1 g of biomass decreased at elevated pCO_2. Effects were largest for those species that had the largest increases in TNC accumulation and the largest decreases in protein concentration (Fig. 4). On average, total construction costs, calculated from the concentrations of C, minerals and organic N, decreased by 3% when plants were grown in high pCO_2 (Fig. 2a). When costs of construction were calculated on a TNC-free dry weight basis, differences were smaller, but still significant (Fig. 2b).

DISCUSSION

Chemical composition

The most pronounced change in the proximate chemical composition of plants grown at high pCO_2 is the increase in total non-structural carbohydrates (Fig. 1; Table 2). However, there is considerable variation in the response of different species, with increases ranging from almost zero to over 100% (Fig. 1). Such increases are comparable to values reported in the literature (cf. Wong 1990; Körner & Miglietta 1994). In the present experiment, we deliberately analysed samples of species which were not grown under exactly the same conditions. This enables us to make more general predictions about the range of changes which is to be expected. A consequence of this approach is that we cannot exclude the possibility that the differences we observed between species are in fact caused by variation in growth conditions. However, in the only large-scale experiment investigating the effects of high pCO_2 on TNC concentrations that we know of (Körner, Pelaez-Riedl & Van Bel 1995), in which 28 dicots grown under standardized conditions were studied, the range in non-structural carbohydrate stimulation (-10 to 130%) was almost as large as for the present 27 species.

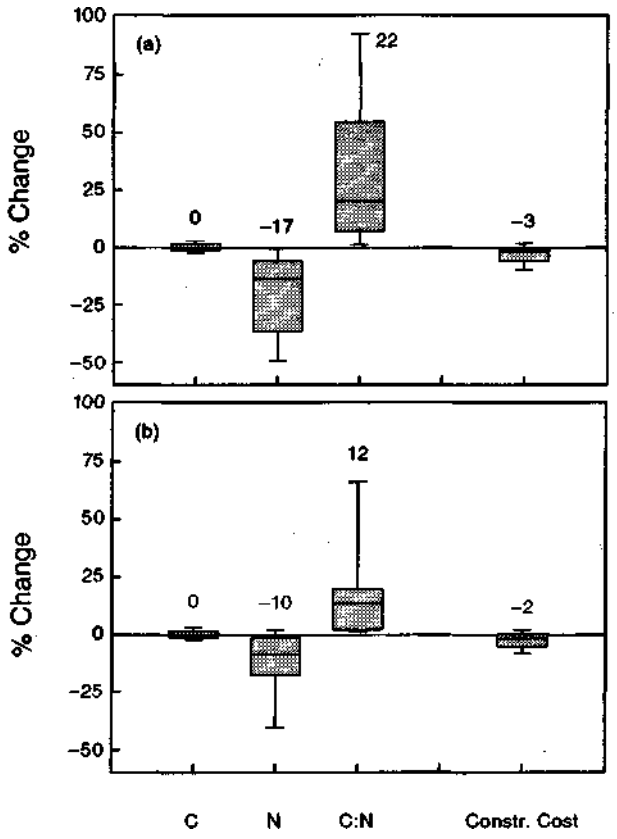

Figure 2. Changes in total C and N concentrations, C/N ratio and construction costs of leaves grown at elevated CO_2. (a) Values on a total dry weight basis; (b) values on a TNC-free weight basis. For further information see Fig. 1.

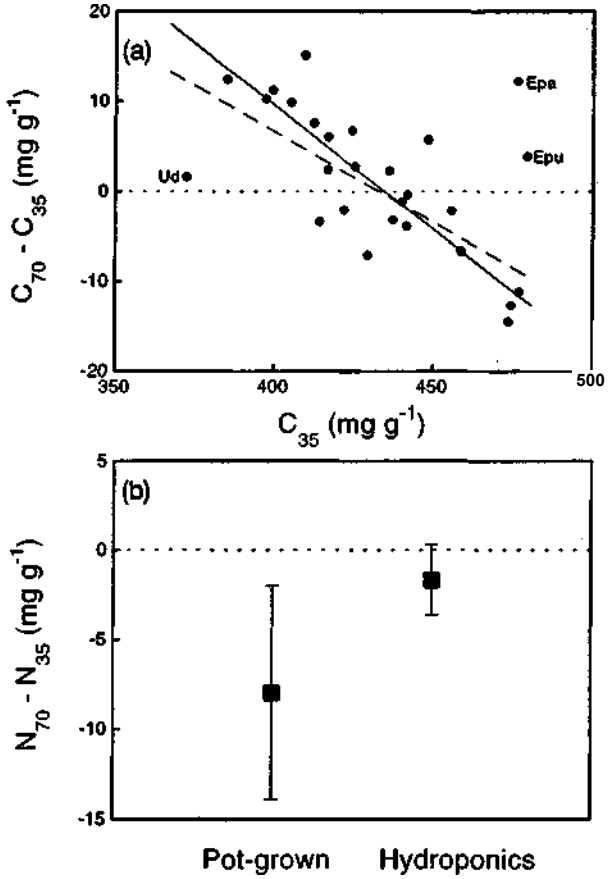

Figure 3. (a) The absolute change in the C concentration (values at 70 Pa - values at 35 Pa) of leaves of 27 species, plotted against the C concentration at control levels of CO_2. The continuous line is the regression line through the data points ($r^2 = 0.71$, $P < 0.001$), with the exception of three outlying species (Epa: *Eucalyptus pauciflora;* Epu: *E. pulverulenta;* Ud: *Urtica dioica).* The dashed line shows the same relationship, after correction for the TNC in the leaf material. (b) The absolute change in leaf total N concentration (on a TNC-free weight basis) for species grown in pots with soil ($n = 19$) and in hydroponics ($n = 8$). Mean values ± SD are shown.

What causes such a large interspecific variation in TNC accumulation? Körner *et al.* (1995) observed a difference in accumulation between species with a symplastic and apoplastic phloem-loading pathway, in that the TNC concentration in symplastic loaders increased less than that in species loading apoplastically. However, this difference was not statistically significant. Bearing in mind the above-mentioned limitation to our analysis, we checked whether we could observe similar changes for those species that could be classified as having a specific type of loading (A.J.E. van Bel, personal communication; see Table 1). Although the sample size was small, we found a significant difference between species with the two types of loading (100% for symplastic loaders, 44% for apoplastic loaders; $P < 0.05$), which is in the opposite direction to that observed by Körner *et al.* (1995). Clearly, this interesting link between loading type and TNC accumulation warrants more investigation. There was no systematic effect of CO_2 on the ratio of soluble versus non-soluble sugars (data not shown).

The second-largest change was the decrease in protein concentration (Table 1). Such observations have been reported often (e.g. Conroy 1992; Coleman, McConnaughy & Bazzaz 1993), but generally it is not clear whether the reported decreases in protein were just the result of a dilution effect of TNC accumulation or a separate phenomenon (but see Körner & Miglietta 1994; Roumet *et al.* 1996). In the present data set, there is a strong negative correlation ($P < 0.001$) across species between the increase in TNC and the decrease in protein. However, from the data in Fig. lb, which are corrected for TNC, we conclude that the decrease in leaf protein concentration is in part an independent phenomenon. What could be the explanation for such a decrease? Testing effects on whole-plant nitrogen concentration, Coleman *et al.* (1993) suggested that the decrease was due to ontogenetic effects, caused by accelerated growth of the high-CO_2 plants. For the sake of simplicity, we have restricted ourselves to one harvest time per species. Therefore, we cannot test this hypothesis. In contrast to the data of Coleman *et al.,* Roumet *et al.* (1996) observed a decrease in N concentration when considering 11 species at a common dry weight.

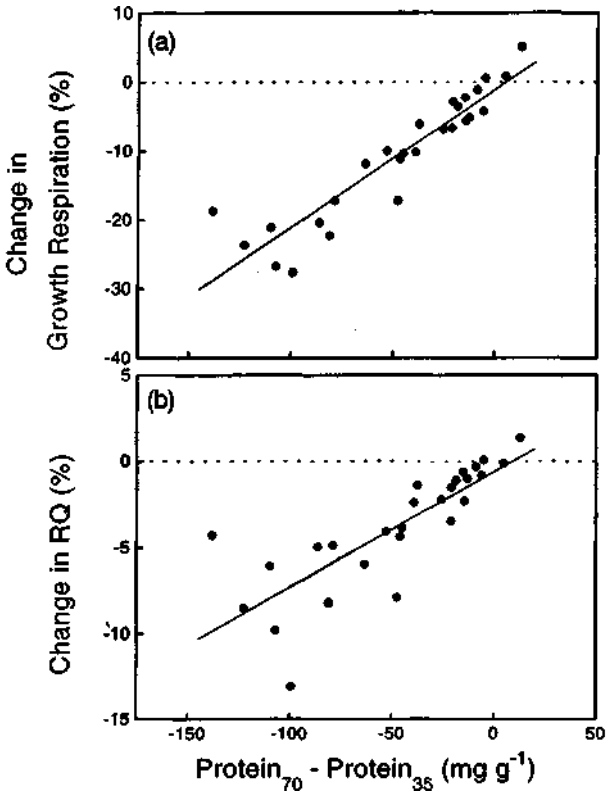

Figure 4. Effect of a twice-ambient pCO_2 on (a) specific growth respiration, and (b) the respiratory quotient of gas exchange related to the growth process (RQ_g), as dependent on the absolute decrease in protein concentration. Data points indicate the percentual change relative to the value at 35 Pa.

Moreover, it is clear that differences in ontogenetic development cannot explain all changes in the chemical composition of high-CO_2 plants. For example, the ontogenetic trend in TNC in plants is quite different from the dramatic increase observed in high-CO_2 grown plants (cf. Baxter *et al.* 1995). Lastly, changes in N at the leaf level are more pronounced than those in stems and roots (Roumet *et al.* 1996; H. Poorter, unpublished results). Therefore, we are not sure that the effect of accelerated ontogeny on chemical composition is a general phenomenon. It would be of interest to analyse whether the observed trends in the study of Coleman *et al.* (1993) still hold when the chemical composition is considered for leaves only, and corrected for both ontogeny and TNC accumulation.

There is an intriguing difference between those species which were grown hydroponically and those grown in pots, in that the former group showed smaller decreases in both organic and total leaf N (Fig. 3b). Two hypotheses have been put forward to explain mechanistically the decreased N availability at elevated CO_2. First, it could be that the decrease in stomatal conductance due to elevated CO_2 (Morison 1987) causes a decrease in the mass flow of ions (especially nitrate and potassium) to the roots, and that this caused pot-grown high-CO_2 plants to take up less nutrients (Conroy 1992). Secondly, elevated CO_2 may increase exudation of C compounds by the roots. This could result in immobilization of N around the roots by the microflora, which profits from the increased root exudation (cf. Diaz *et al.* 1993). Neither mechanism is likely to affect N uptake in well-stirred hydroponics and thus could offer an explanation for the different behaviour of plants grown in different media. However, these mechanisms will only work if nutrient supply in pots is approaching limitation anyway. For highly mobile ions like nitrate, uptake by the roots in well-watered pots which are supplied with high amounts of nutrients and water is generally not limited by mass flow (cf. Strebel & Duynsveld 1989). Similarly, immobilization of N by microbial biomass occurs, but is quantitatively negligible compared to total N in the biomass (Schenk *et al.* 1995). By rejecting these two hypotheses, we presume that the primary cause of the stronger decrease in leaf N in pot-grown species as compared to hydroponically grown species is the lower availability of N in pots.

The only other group of compounds that was affected by elevated CO_2 was the soluble phenolics. They increased by an average value of 17% on a TNC-free basis. A similar stimulation was reported by Cipollini, Drake & Whigham (1993). Lambers (1993) suggested that increases in phenolics could be an indirect effect of the decrease in organic N compounds, rather than a direct effect of high CO_2. We tested this hypothesis by analysing the relationship between the proportional increase in phenolics and the proportional decrease in protein, both on a TNC-free dry weight basis (data not shown). There was indeed a significant positive relationship between these two parameters. However, r^2 was low (0.15) and the relationship hinged on one of the eucalypt species, suggesting that this hypothesis is not generally valid.

Little is known about the effect of elevated CO_2 on lignin. It has been measured a number of times in senesced leaves from decomposition studies, with reports of both increases (Cotrufo, Ineson & Rowland 1994) and decreases (Gifford *et al.* 1995). Concentrations of lignin in green leaves has received less attention. We did not find much of a difference when concentrations were expressed on a dry weight basis, consistent with data of Kemp *et al.* (1994).

On average, total C concentration was not affected by high CO_2. However, this does not imply that individual plant species were not altered. With the exception of three species, there was a negative relationship between the absolute change in C concentration due to high CO_2, and the C concentration in the leaves at control levels. That is, species with a normally low C concentration increase in C in response to elevated CO_2, whereas those with a high C concentration decrease in C (Fig. 3a). A similar observation was made by Poorter *et al.* (1992), who compiled data from a wide variety of sources. They considered this phenomenon to be due to starch accumulation. Starch has a C concentration of 440 mg g^{-1}. Thus, one would expect plants with concentrations below that value to increase their C concentration upon starch accumulation, and those with values above 440 mg g^{-1} to decrease in C concentration. Figure 3a demonstrates that this is the case. However, although qualitatively correct, the observed negative relationship does not disappear when the change in C concentration is calculated on a TNC-free basis (see dotted line in Fig. 3a). What, then, is the main reason for the observed negative relationship? Species with a low C concentration are generally those with a high mineral concentration, which is in fact the main reason that C is low in those species. As high CO_2 decreases the mineral concentration to a greater extent in low-C plants than in high-C ones, this is the main reason for the observed negative relationship. Apart from changes in these two compounds, the decrease in lipids also plays a small role. The decrease in lipids is greater for plants with a high C concentration ($P < 0.05$). As lipids are high in C, the decrease in C at elevated CO_2 is also partly attributable to the change in lipids. Thus, the fact that the cross-over point in Fig. 3a corresponds to the C concentration of starch is partly fortuitous. Not all species follow the general trend. Two of the *Eucalyptus* species, for example, do have high concentrations of C and nevertheless increase their carbon concentration in response to elevated CO_2. This may imply that, in response to an increase in pCO_2, such species are capable of producing one or more secondary compounds with a high C concentration. One of these species was found to show a large increase in soluble phenolics, and both increased in lignin concentration.

Growth respiration

A topic that has raised discussion in the literature is the effect of CO_2 on respiration. Both increases and decreases in leaf respiration have been reported, as well as no effect (for reviews, see Poorter *et al.* 1992; Amthor 1996). With

the purpose of obtaining a more mechanistic understanding, respiration has been separated into a maintenance and a growth component (see e.g. Thornley 1970). Growth respiration can be derived from the changes in respiration with changes in growth rate. In soybean and cotton, increases have been found in maintenance respiration, whereas specific growth respiration, the respiration associated with the production of 1 g of biomass, was reported not to be affected by elevated CO_2 (Thomas *et al.* 1993; Thomas & Griffin 1994; Bunce 1995). On the other hand, Wullschleger & Norby (1992) and Wullschleger, Norby & Gunderson (1992) found maintenance as well as specific growth respiration to be decreased, though not always significantly, in leaves of two tree species. Using the present data we can make an independent estimate of the respiration associated with growth, based on chemical composition, following Penning de Vries *et al.* (1974). As we could not account for 100% of the leaf material, but did not observe systematic differences between control- and high-CO_2 grown plants (Appendix 1), we assumed the remainder to be TSC. On average, there was an 11% decrease in the calculated growth respiration (on the basis of CO_2 production) due to growth at elevated carbon dioxide, with values ranging from +4 to -27%. The magnitude of this decrease corresponds well to the above-cited regression estimates. This decline correlated with both the accumulation of TNC and the reduction in protein (Fig. 4a, $P < 0.001$, $r^2 = 0.86$). The accumulation of TNC has a direct effect on growth respiration, as compounds like starch can be formed with little CO_2 production. The reduction in protein also has an effect, as protein is a class of compounds whose synthesis is accompanied by a large CO_2 production. To discriminate between the two processes, we recalculated specific growth respiration on the basis of TNC-free dry weight, protein-free dry weight and corrected for both compounds. The reduction in protein concentration is more important than the increase in TNC in explaining the decreased growth respiration per unit of biomass formed, being responsible for 60% of the total decrease (Fig. 5). Apart from the effects of protein and minerals, there are also small effects of lipids, organic acids and minerals, which counteract each other.

In the above calculations of growth respiration, we assumed that NO_3^- was the N source of the plants, and that the reducing power required for the transformation of nitrate to ammonium is derived from glucose rather than drawn directly from excess reducing power in photosynthesis. In leaves, this is likely not to be the case, although the exact contributions of the two sources are unknown. If the above assumption were correct, with a decrease in protein concentration, there would be less demand for NAD(P)H (to reduce nitrate) than for ATP. In that case, growth-related CO_2 production would decrease to a larger extent than O_2 consumption. Indeed, the respiratory quotient of growth respiration (RQ_g) decreases. This decrease is correlated with the reduction in protein concentration (Fig. 4b, $P < 0.001$, $r^2 = 0.65$) and ranges between 0 and 10%.

Construction costs

Construction costs are defined as the amount of glucose required to build 1 g of biomass. This comprises both the C necessary for C skeletons and the glucose required for NAD(P)H and ATP production to drive the various biosynthetic reactions (Penning de Vries *et al.* 1974). On average, C concentration does not change due to high CO_2, whereas growth respiration decreases on average by 11%. Given that the growth respiration component represents *c.* one-third of the total leaf construction costs (0.43 g glucose g^{-1} DW out of a total of 1.50 g glu g^{-1} DW; Poorter & Villar 1997), we would expect the construction costs at high CO_2 to decrease by 3-4%. This is in full agreement with the change in construction costs, as determined independently from C, mineral and organic N concentration. The decrease is consistent with observations of Griffin, Thomas & Strain (1993) for *Pinus taeda,* and Amthor *et al.* (1994) for *Glycine max,* who also found rather minor changes. For C_4 species, in which smaller effects of CO_2 on chemical composition are expected, no decreases in construction costs were found (Loomis & Lafitte 1987; Amthor *et al.* 1994).

Generally, plant growth responds far less to high pCO_2 than expected on the basis of A-p_i curves (Poorter 1993).

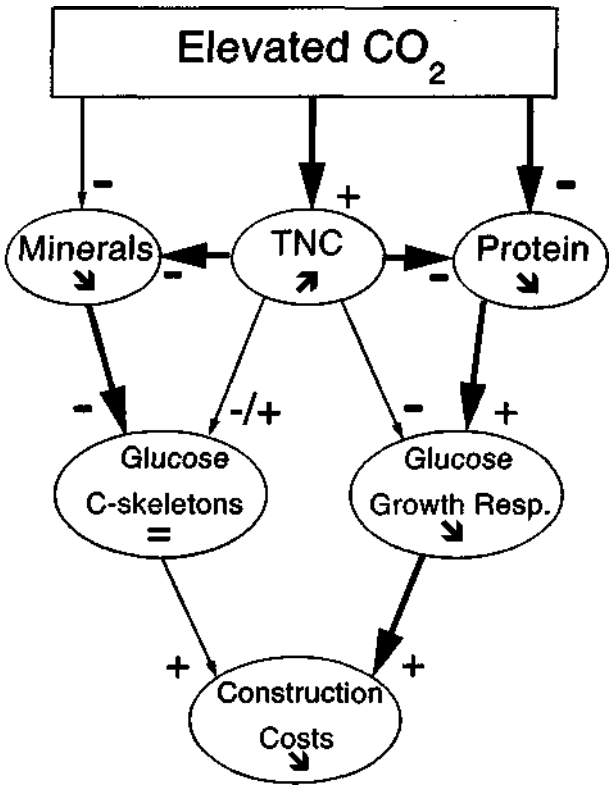

Figure 5. Schematic representation of the most important changes in chemical composition of plants at elevated CO_2, and the consequences for leaf construction costs and its components. Thick arrows represent strong effects, and thin arrows smaller effects. A '+' indicates a positive effect, and a '-' a negative one. The net result of all the effects on the various constituents and glucose costs is indicated by the up and down arrows in the boxes.

As mentioned in the Introduction, part of this modest response might result from secondary changes, such as changes in biomass allocation or construction costs. From the present data, and the scarce literature, we expect such changes to be marginal. In those species where construction costs decrease by a larger extent (a decrease of 10% yields an increase in relative growth rate of 10%, all other things being equal; Poorter & Villar 1997), growth stimulation is expected to be larger rather than smaller. Therefore, we conclude that a direct effect of high CO_2 on the chemical composition of the plants is not likely to affect the carbon balance of the plant to a large extent. If any effect is present, the tendency seems to be for an increase in growth stimulation, rather than a decrease. However, the increase in TNC at elevated CO_2 may indicate that carbon is not the limiting factor for high-CO_2 grown plants. It would be of interest to determine whether variation in TNC accumulation scales negatively with the growth response of different species to elevated CO_2.

Conclusions

A twice-ambient CO_2 partial pressure affected the chemical composition of leaves mainly through the accumulation of TNC. Independent of this accumulation there was an increase in the concentration of soluble phenolics and a decrease in the concentration of protein and minerals. Other compounds were only slightly affected. There was a slight decrease in the estimated growth respiration, and, consequently, also a decrease in the construction costs of the leaves.

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APPENDIX 1

Table A1. Leaf chemical composition of 27 species grown at control (c; 35 Pa) and elevated (e; 70 Pa) pCO_2. All data are average concentrations determined on two independent bulk samples. Values are expressed on a dry weight basis, in mg g^{-1}

	Lipids		Sol. Phenol.		Protein		Lignin		TSC		TNC		Org. Acids		Minerals		Total	
Species	c	e	c	e	c	e	c	e	c	e	c	e	c	e	c	e	c	e
Crop																		
B. pekinensis	57	52	15	15	295	249	17	13	129	132	64	126	149	123	170	129	895	839
C. sativus	51	51	6	8	338	215	6	5	154	111	71	303	196	130	128	93	950	916
G. max	48	47	12	12	237	191	28	34	120	101	278	316	92	85	42	36	859	823
G. hirsutum	48	32	9	9	251	152	46	43	130	121	94	305	163	129	115	70	857	860
L. perenne	47	46	18	18	331	316	58	63	111	115	110	129	42	31	131	130	848	848
M. sativa	56	50	7	7	346	321	30	21	95	104	182	242	71	66	86	71	873	881
P. sativum	47	37	21	16	187	140	16	14	96	85	384	436	80	63	49	40	881	832
S. tuberosum	62	64	11	14	311	290	36	44	115	113	121	170	84	91	137	108	877	894
T. aestivum	58	60	11	13	218	231	14	20	298	290	107	101	19	19	136	133	862	866
Wild herbaceous																		
A. theophrasti	48	34	14	19	294	186	36	21	113	88	207	389	78	60	79	58	870	856
A. capillaris	63	53	22	26	315	236	49	47	208	210	126	206	27	22	68	51	878	851
B. erectus	46	44	32	34	300	288	44	27	158	161	106	141	47	58	137	130	869	883
B. hordaceus	47	50	14	16	333	296	18	32	172	128	92	158	58	61	144	143	878	885
B. madritensis	54	51	33	35	320	281	44	31	127	119	106	189	52	65	146	124	883	895
B. tectorum	50	48	28	33	305	300	62	73	150	126	93	96	68	68	124	124	879	868
E. crassipes	56	56	10	9	324	329	44	39	120	144	100	112	96	96	123	115	874	900
F. vivipara	43	41	11	10	295	242	65	87	219	218	110	130	19	20	72	59	836	808
T. subterraneum	72	73	24	25	275	257	26	25	133	121	142	193	100	98	73	61	845	854
P. major	48	42	6	7	233	212	10	10	141	143	176	228	93	91	162	131	869	863
U. dioica	24	28	2	2	307	298	na	na	140	171	146	154	265	262	123	118	1008	1012
Woody																		
A. auriculiformis	54	50	76	73	250	244	84	70	206	176	118	196	63	66	54	50	904	925
A. melanoxylon	67	56	18	31	365	255	64	68	112	76	122	240	62	83	57	43	868	853
E. camaldulensis	60	53	75	81	159	78	66	55	107	100	240	351	42	34	39	25	788	776
E. cypellocarpa	118	81	102	144	167	81	68	50	111	96	117	244	54	46	45	34	781	776
E. pauciflora	80	86	102	82	150	86	90	127	162	158	113	209	52	36	37	21	786	805
E. pulverulenta	113	124	34	70	250	111	85	98	146	136	93	214	51	42	47	36	818	831
V. vinifera	59	55	34	30	213	199	57	53	117	119	222	277	64	61	51	41	817	834