

Carbon limitation in trees

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Summary

1 The ongoing enrichment of the atmosphere with CO₂ raises the question of whether growth of forest trees, which represent close to 90% of the global biomass carbon, is still carbon limited at current concentrations of close to 370 p.p.m. As photosynthesis of C3 plants is not CO₂-saturated at such concentrations, enhanced 'source activity' of leaves could stimulate 'sink activity' (i.e. growth) of plants, provided other resources and developmental controls permit. I explore current levels of non-structural carbon in trees in natural forests in order to estimate the potential for a carbon-driven stimulation of growth.

2 The concentration of non-structural carbohydrates (NSC) in tree tissues is considered a measure of carbon shortage or surplus for growth. A periodic reduction of NSC pools indicates either that carbon demand exceeds con-current supply, or that both source and sink activity are low. A steady, very high NSC concentration is likely to indicate that photosynthesis fully meets, or even exceeds, that needed for growth (surplus assimilates accumulate).

3 The analysis presented here considers data for mature trees in four climatic zones: the high elevation treeline (in Mexico, the Alps and Northern Sweden), a temperate lowland forest of central Europe, Mediterranean sclerophyllous woodland and a semi-deciduous tropical forest in Panama.

4 In all four climatic regions, periods of reduced or zero growth show maximum C-loading of trees (source activity exceeding demand), except for dry midsummer in the Mediterranean. NSC pools are generally high throughout the year, and are not significantly affected by mass fruiting episodes.

5 It is concluded that, irrespective of the reason for its periodic cessation, growth does not seem to be limited by carbon supply. Instead, in all the cases examined, sink activity and its direct control by the environment or developmental constraints, restricts biomass production of trees under current ambient CO₂ concentrations.

6 The current carbohydrate charging of mature wild trees from the tropics to the cold limit of tree growth suggests that little (if any) leeway exists for further CO₂-fertilization effects on growth.

Key-words: carbohydrates, climate, elevated CO₂, forest growth, latitude, season, storage

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Trees in an increasingly CO₂ enriched world

Among the unquestioned components of global change, atmospheric CO₂ enrichment holds a special position; first, because it has no spatial restriction and reaches every place on earth, and secondly, because it directly affects the most elementary life process,

photosynthesis of green plants. Given that the capacity of the photosynthetic machinery of C3-plants remains unsaturated at current concentrations of close to 370 p.p.m. CO₂, there is a potential for a carbon-driven stimulation of plant growth now and, perhaps, in an even more CO₂-rich future. The current CO₂ concentration is 30% above the pre-industrial concentration and twice as high as it was only *c.* 18 000 years ago, during the last glaciation (Neftel *et al.* 1988). Over the preceding 400 000 years, CO₂ concentration had never exceeded 290 p.p.m. (Petit *et al.* 1999).

Although a change in the rate of growth should not be mistaken as an indication of a change in carbon pool size (Steffen *et al.* 1998; Körner 2000; Schulze *et al.* 2000), it is still of interest to know whether there is any leeway for pumping more carbon through the globe's biota. A transitory accumulation of C in biomass could slow atmospheric CO₂-enrichment (thereby 'buying time') and thus help society in its development of mitigation measures before the system approaches a new steady state with net biosphere carbon balance close to zero. Currently 1–2 Gt C a⁻¹ (c. 10–20 g C m⁻² of vegetated land) are missing in the globe's carbon balance, and are most likely captured by terrestrial biota (e.g. Falkowski *et al.* 2000; IPCC 2001).

Trees store close to 90% of all biomass carbon on earth (c. 500 Gt C), and this pool is not so different in size from the total amount of C in the atmosphere (750 Gt C). Atmospheric C-content is therefore sensitive to forest destruction, and potentially also to forest biomass increments (for references see Körner 2000). Are forests likely to be stimulated by the continually increasing availability of CO₂, now and in a distant future? Although forests play such a prominent role in the global C-cycling, their response to elevated CO₂ is largely unknown, because the sheer size of maturing or adult trees constrains conventional growth analysis under experimental CO₂ enrichment.

To date, we have data from only two open air CO₂-enrichment experiments with closed tree stands at or near to reproductive age: a 17-year-old *Pinus taeda* forest in North Carolina, and a 12-year-old *Liquidambar styraciflua* stand in Tennessee. These rapidly growing plantations showed pronounced growth stimulations during the 1–3 years following a step increase in CO₂ concentration by c. 200 p.p.m. (e.g. DeLucia *et al.* 1999; Norby *et al.* 2001). However, there is a possibility that these initial responses will not be sustained (Norby *et al.* 2001; Oren *et al.* 2001). *Pinus taeda* also exhibited accelerated maturation (earlier and more massive production of cones; LaDeau & Clark 2001), which hints at the possibility of enhanced forest turnover in response to CO₂-enrichment. It became obvious from studies with open top chambers and other enclosure techniques (cf. Lee & Jarvis 1995), that a stimulation of leaf level photosynthesis is not a good predictor of growth responses in stands that have arrived at canopy closure. Extra photosynthates may pass through channels other than new structural biomass, for instance enhanced respiratory output, as was observed earlier in smaller scale trials (e.g. Körner & Arnone 1992; Hättenschwiler & Körner 1996).

The major drawback of all CO₂-enrichment experiments, irrespective of the enrichment method, is the compound interest effects on growth following initial responses to a step increase in CO₂-concentration. Once an initial difference is induced, it needs no further stimulation for the signal size to increase with time (Körner 1995; Norby *et al.* 1995; Tissue *et al.* 1997; 2001; Spinnler *et al.* 2002). Hence, there is a chance

that signals produced by a doubling CO₂-treatment are not representative of the effect of a long-term 1–2 p.p.m. increase in CO₂ per year (Luo & Reynolds 1999). Based on tree rings, annual growth of even aged Mediterranean evergreen oak around two natural CO₂-springs was stimulated initially, but came down to control rates in 25 years (despite compound interest effects of initial gains in young stands), in large because of a reduction of the trees' relative investment in leaf area (Hättenschwiler *et al.* 1997a,b; see the below paragraph on Mediterranean trees). Various mature tree species cored at only one of these sites, were also not found to respond significantly to elevated CO₂ (Tognetti *et al.* 2000).

A given CO₂-increment in the current range of CO₂ concentrations, which are, physiologically, still low, should therefore be more effective than a similar one at future higher concentrations. Therefore forests should exhibit significant growth responses right now, if CO₂ is a rate-limiting resource. Two types of studies may assist in documenting such ongoing trends: (i) assessments of net ecosystem productivity of natural forests by CO₂-flux measurement; and (ii) the long-term study of tree demography from which transition probabilities across a range of tree age classes can be derived. Flux measurements have indicated the possibility of currently enhanced net C-binding (e.g. Grace *et al.* 1995), but their intrinsic problem is that any non-senescent forest should fix C, otherwise it would not grow. Hence very large areas need to be covered to include all life stages in a representative way, an avenue now chosen by a global consortium collecting eddy flux data (cf. Canadell *et al.* 2000). Forest inventories, on the other hand, are only powerful in unmanaged areas, far away from immediate pollution (N-deposition), need very long time series and once more, a full coverage of tree age classes. Such assessments in the tropics seem to indicate the possibility of enhanced tree turnover in recent decades (e.g. Phillips *et al.* 1998), but this may well be the result of more aggressive growth of climbers (Granados & Körner 2002; Phillips *et al.* 2002), rather than a direct stimulation of tree growth (atmospheric CO₂-enrichment being relatively more effective in deep shade, where climbers start off).

It will take a while before we will arrive at a conclusive picture of CO₂-enrichment effects representative of the majority of the world's forests. One hundred to 120-year-old deciduous forest trees (*Quercus*, *Fagus*, etc.) have now been exposed to elevated CO₂ in Switzerland for two seasons (Pepin & Körner 2002), but it is too early to draw conclusions about growth responses to CO₂-enrichment under such natural growth conditions.

Here, I offer an approach, which has not previously been explored systematically, namely an assessment of the carbon supply status of wild trees based on the size and periodic change of the mobile C-pool in tissues. This approach also has its limitations and does not provide any direct evidence of a current or possible future growth response to greater availability of carbon

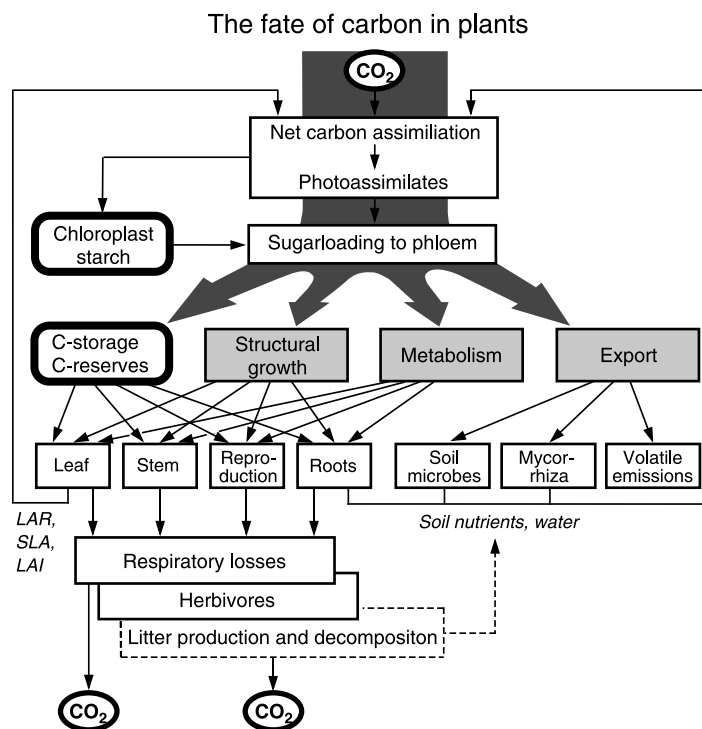


Fig. 1 A schematic representation of the fate of carbon in plants. The size of mobile C-pools indicated to the left, reflects a tree's carbon supply status (LAR, leaf area ratio; SLA, specific leaf area; LAI, leaf area index).

assimilates to trees. However, it provides circumstantial evidence of the likelihood of enhanced C-uptake, and it is not constrained to certain experimental conditions, but permits the study of natural forests anywhere.

A sensor of a tree's carbon balance

A tree's biomass gain is the net outcome of assimilatory carbon input (net photosynthesis of leaves) minus carbon output (respiration, losses of dry matter, various forms of organic C-export; Fig. 1). The biomass gain may be separated into a C-pool, which also ties up elements other than C, O and H (enzymes, proteins and peptides, non-COH secondary compounds, cell wall material, cell organelles), and a mobile 'pure' COH pool, not including other elements, in essence the mobile organic C fraction composed of carbohydrates, sugar alcohols, organic acids, lipids, phenolics, isoprene polymers, terpenoids, etc. The largest part of this latter fraction commonly falls into non-structural carbohydrates (NSC) and lipids. This potentially mobile pool represents either carbon reserves or carbon storage (Chapin *et al.* 1990). Its size may be considered an indicator of a plant's 'fuelling' status with respect to C, because it should reflect any shortage (depletion) or surplus (accumulation), i.e. the balance of C-source vs. C-sink activity (Fig. 1). This assessment will use NSC (and in some cases also lipids) in order to infer the degree of carbon limitation at a whole tree basis in the course of the year, across a wide range of climates.

The NSC assay used throughout this multiyear survey, involves fractionated hot water extraction of

powdered plant material, enzymatic breakdown of starch and disaccharides followed by a photometric analysis of the resultant glucose using a hexokinase reaction kit. Plant samples were randomly checked for the abundance of other mobile COH compounds using a gas chromatograph, but these compounds (except for conifer sapwood) never accounted for more than 30%, mostly far less (< 15%), of all mobile COH-compounds. In sapwood tissue of conifers, lipids can account for 50–70% of all non-structural carbon and were quantified as glycerol equivalents using a photometric analysis of fat-bound glycerol after saponification of acylglycerols and an enzymatic conversion of glycerol (for details on methods see Hoch *et al.* 2002).

Mobile C-pools in trees of four climates

In the following four sections I will compare the mobile C-pool in trees during favourable and less favourable periods of the year in four climatic zones. One might expect adverse environmental conditions to induce a depletion of C-pools (plants falling short in C-assimilates), whereas pools should be large and/or be re-charged during more favourable conditions. This rationale assumes a tight coupling between photosynthesis, the size of the mobile C-pool and structural growth in this sequence of effects. I will, however, argue that the sequence of effects is actually reversed, namely that the predominant control of photosynthesis comes from growth (carbon demand, sink activity), and that NSC in tissues indicates the degree to which growth is C-limited.

Each section will start with a description of the current natural seasonal variation in NSC (or lipids), followed by a brief account of examples illustrating how NSC stores and growth of trees respond to an experimental increase of elevated CO₂ (commonly a doubling of the pre-industrial or current concentration). The more CO₂-enrichment increases the non-structural C-pool, the less likely carbon is to be a growth limiting resource.

TREES AT THEIR LOW TEMPERATURE LIMIT

I will first explore the extent to which the increasingly short, cold growing season at treeline elevations in Mexico, the Alps and Northern Sweden (i.e. the cold end of tree distributions) constrain a tree's ability to maintain an adequate C-supply. Transects are located at Pico de Orizaba, at 20° N in Mexico with a 7–8-month season, the Swiss central Alps at 46° N with a 5-month season, and the northern Scandes at 68° N with a 2–3-month season. In all three cases pines form the natural high elevation treeline (at *c.* 4000 m, 2350 m and 420 m altitude). Given the rather abrupt termination of upright tree existence over a very short distance, with tall trees found at only 100–150 m lower elevation, it is tempting to assume a thermal threshold phenomenon (Körner 1998, 1999). If this threshold is associated with carbon assimilation, a dramatic depletion of NSC should occur across the ecotone towards the tree limit.

In this specific case, involving a spatial climatic gradient, the use of a dry matter-based presentation of NSC (%) is problematic, as tissues get denser as one approaches the tree limit (e.g. narrower tree rings, lower specific area of needles, SLA). In other words, any elevational trends in NSC could reflect either a 'true' NSC difference or a difference in tissue density, with little change in NSC content per active cell. Therefore, data will be discussed also with reference to tissue volume (wood) and area (needles).

Counter to expectation, NSC in sapwood increases with elevation on both a dry matter and, even more so, on a volume basis (Fig. 2 shows the dry mass data). Similar trends are found in needles, branches and roots (Hoch *et al.* 2002; Hoch & Körner 2003). Lipid concentrations in woody tissue also peak at the tree limit. Osmotically active compounds increase as well, but their quantitative contribution to the overall trend is small. There was no significant difference between the three locations, despite the more than twofold difference in season length. Equally surprising, the seasonal course of NSC concentrations in needles peaks at the end of winter, right at the onset of the growing season, suggesting that these evergreen trees assimilate a lot of carbon during the pre-growth, late winter period. Flushing itself hardly affects NSC, suggesting either that the expanding shoots are self-supporting or that they are fed directly via ongoing photosynthesis in older generations of needles, as was shown to be the

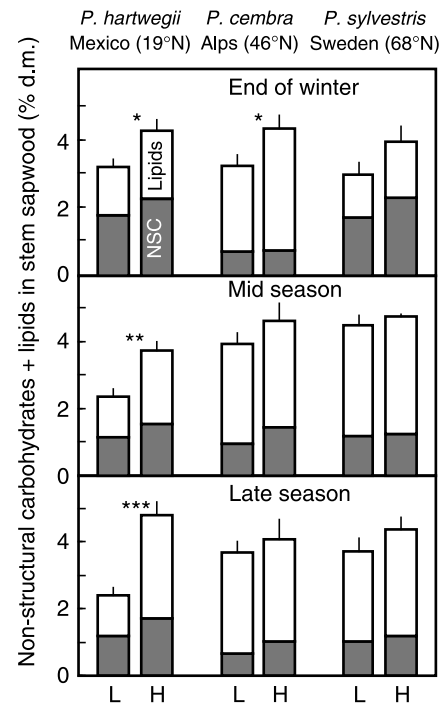


Fig. 2 Non-structural carbohydrate and lipid concentrations in sapwood of pine trees at the upper elevational tree limit (H) and at *c.* 100–150 m lower elevation (L) in three climatic zones (from Hoch & Körner 2003).

case in lowland *Pinus sylvestris* by Hansen & Beck (1994). In any case early season growth does not cause a net drain of reserves (cf. Oleksyn *et al.* 2000 and the early work by Wight 1933, for *P. sylvestris*). Apart from a small (insignificant) midseason depression, trees enter the dormant season as packed with NSC as when growth started. A massive experimental defoliation at the beginning of the treeline season in the Alps caused a significant mid-season NSC depletion, illustrating that NSC pools are sensitive to such a major disturbance of the source-sink balance. Nevertheless, by the end of the season, pools had almost recovered, despite the restriction of assimilation to only one (new) needle generation (Li *et al.* 2002).

All in all, neither the ecotone transect, nor latitudinal or temporal differences, nor the defoliation data support the idea of carbon limitation of growth at these treelines. Data for mountain birch of Northern Scandinavia point in the same direction (Mäenpää *et al.* 2001 and earlier works by Skre 1993). NSC increases in birch with altitude and when sugars and starch for perennial tissues are pooled, the seasonality of carbon reserves mentioned by these authors actually disappears. From the data for the three pine treelines, one gets the impression of a 'full tank' situation in which the activity of sinks rather than that of sources (photosynthesis) limits growth (cf. Körner 1998). As for low stature alpine plants (Körner 1999), growth of treeline trees appears to be cold-limited by processes involved in tissue formation rather than by photo-assimilate provision.

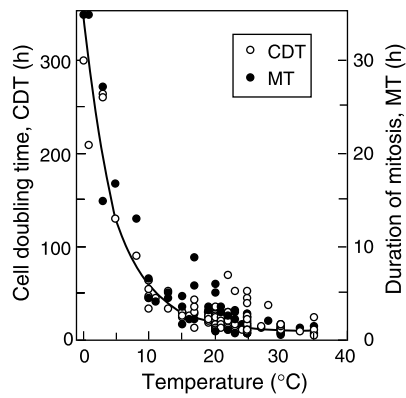


Fig. 3 A literature survey of the dependency of cell division on temperature, expressed as either cell doubling time or mitotic time (70 references, Körner 1999; reproduced with permission by Springer Verlag, Heidelberg).

The formation of new cells is much more sensitive to low temperature than photosynthesis. Cell doubling time, for instance, approaches infinity at +1 to +2 °C (Fig. 3), even in cold adapted plants, whereas the rate of photosynthesis in treeline trees may still be 30% of full capacity at such temperatures (Tranquillini 1979). Temperatures during the night and during bad weather are commonly close to zero at the treeline and thus, inhibit investment of carbon into structural growth for a substantial fraction of the season. These findings are in line with Schimper's (1898) idea that 'photosynthesis has the lowest "cardinal point" of temperature' (in the sense that most other plant processes become low temperature limited before photosynthesis). In line with this rationale, growth was found to be inhibited, and starch concentrations to be greater in *Pinus sylvestris* seedlings grown at 4 °C rather than at 18 °C (Kontunen-Soppela *et al.* 2002).

Ongoing *in situ* CO₂ enrichment of 30-year-old treeline trees in the Alps (Hättenschwiler *et al.* 2002) revealed a significant increase of NSC in foliage during the first year of treatment (Fig. 4) as was found with bagged branches in 30-year-old Scots pine in Finland (Laitinen *et al.* 2000). Concentrations in branch sapwood increased as well, but the difference was only marginally significant. A 3-year phytotron study with 5–7-year-old montane *Picea abies* model-stands on natural substrate also revealed NSC increases. In response to elevated CO₂ trees showed no above-ground growth stimulation, but had enhanced below-ground respiration and drastically reduced needle mass per stem mass and needle area per ground area (LAI 3.7 instead of 5.2; Hättenschwiler & Körner 1998), similar to observations in Mediterranean oak around CO₂ springs (see later). These spruce trees, grown on montane forest soil, were indeed carbon saturated at current CO₂ concentrations. A continuously enhanced glucose concentration in needles may have signalled restricted carbon demand and led to down-regulation of photosynthesis (Wiemken & Ineichen 2000). Tall spruce

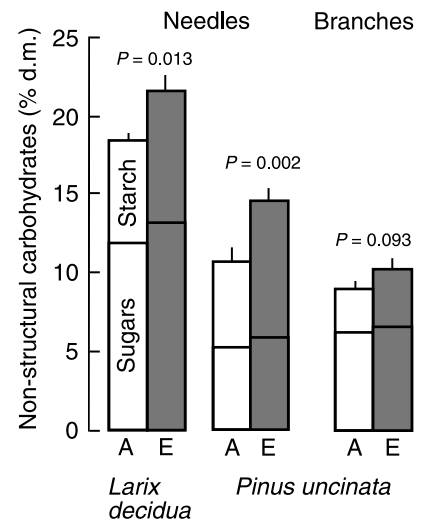


Fig. 4 *In situ* responses of NSC in foliage and branch sapwood of conifers to elevated CO₂ at the Swiss treeline during the first year of treatment in 2001 (Hättenschwiler *et al.* 2002). A and E for trees exposed to ambient and elevated CO₂ concentrations. d.m. = dry matter.

individuals close to the boreal tree limit also showed no significant growth response to CO₂-enrichment (Robertz 1999). Taken together with the experimental data, the continuous high abundance of NSC and lipids, and their increase towards the low temperature tree limit, argue against the 'carbon limitation' hypothesis of treeline formation.

TEMPERATE ZONE FOREST TREES

With a 5–6-month growing season at 47° N and only 1–4% of total biomass in autotrophic structures (Körner 1994), a long, often mild winter should exert a major drain on NSC pools, particularly in deciduous trees. In addition, the spring flush should diminish reserves (Zimmermann & Brown 1974; Kramer & Kozłowski 1979; Höll 1997). Years with heavy fruiting (so called 'masting' years), which commonly occur at intervals of 3–5 years, should also represent a major burden for a tree's mobile C-pool (e.g. Gäumann 1935; Holmgaard 1958 for *Fagus*). We examined these facets of carbon relations in a 35-m tall oak-beech-hornbeam forest near Basel (Hoch 2002).

Stem sapwood cores of each of two trees per species, taken at four dates in the 1999 growing season reveal very little seasonal variation in NSC (time series analysis n.s., except for the second half of the season in *Quercus*) and generally very high concentrations (Fig. 5). However, in branch wood (not in stem sapwood), four out of six species showed significant seasonal changes, with similarly high mean concentrations to those in stems (data not shown). For *Quercus* and *Fagus*, two 'masting' species, branch wood data are available for both 1999 and 2000 (Fig. 6). As either all or none of the trees of a given species mast in a given year, the year-effect

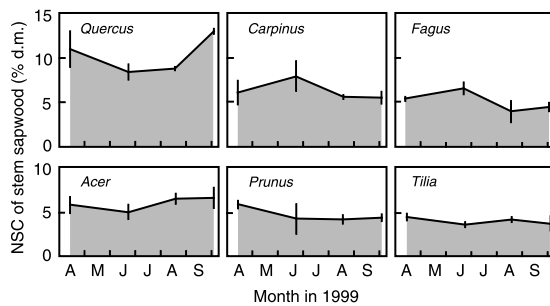


Fig. 5 The seasonal variation in NSC in stem sap wood in adult, 30–35 m tall deciduous forest trees ($n = 2$ trees) near Basel, Switzerland (Swiss canopy crane site; Hoch 2002). d.m. = dry matter.

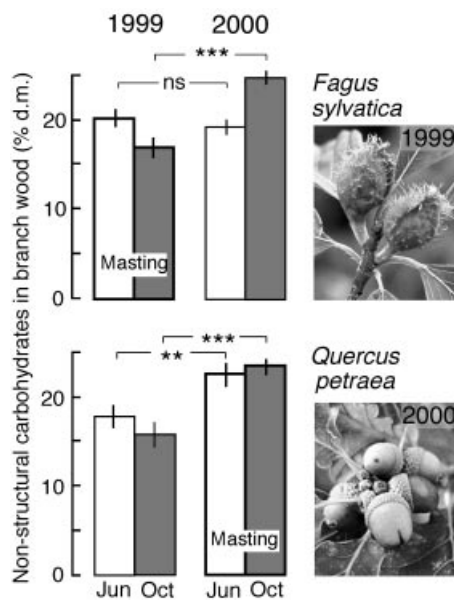


Fig. 6 NSC concentrations in branch wood of masting and non-masting specimens of *Quercus petraea* and *Fagus sylvatica* (Hoch 2002). d.m. = dry matter.

cannot be fully separated from the masting effect. In 1999 both the masting *Fagus* and the non-masting *Quercus* showed a declining trend as the season progressed. In 2000 both species showed an increasing trend. Although neither was, on its own, significant, these opposite trends led to a highly significant year by year variation in the end of season NSC status in both species, irrespective of masting. It is remarkable that branch NSC showed no late season reduction whatsoever in masting *Quercus* in 2000 and was in fact 40% higher than in the previous season when there was no fruit production. These findings are in line with those by Bonicel *et al.* (1987) and Schaberg *et al.* (2000), who also noticed rather stable NSC pools in wood of deciduous trees.

At the given temporal resolution, these data indicate very little dependency of seasonal growth on stored NSC and thus contrast with views based on data mainly collected during the first half of the 20th century (Kramer & Kozłowski 1979). None of the six species tested showed a depletion of NSC towards the end of

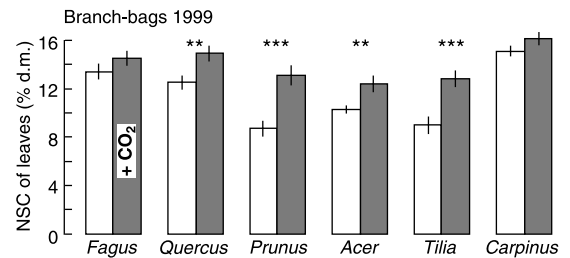


Fig. 7 NSC concentrations in leaves of CO_2 -enriched branchlets (shaded bars) and controls in tall deciduous forest trees near Basel (S. Guillod, S. Pepin, Ch. Körner, unpublished data). d.m. = dry matter.

the dormant period. Flushing did not significantly draw upon stem reserves, or if it did, this must have been a very short-lived effect, not reflected in the data. In a given season, masting did not significantly affect NSC in storage tissue and, in fact, heavily masting *Quercus* showed the highest ever recorded NSC concentration late in the year 2000 season. Taken together, one is left with the impression that these trees never pass through periods in which stores are needed to balance demand. By forestry standards, the test site, which has a basal area of $45 \text{ m}^2 \text{ ha}^{-1}$, is rated as fertile (with annual atmospheric N-deposition around $30 \text{ kg N ha}^{-1} \text{ a}^{-1}$). Hence, trees in a closed stand may be growing at near full capacity for their age and size, but carbon acquisition seems to cope well with this demand. In 10-year-old, warm temperate *Pinus taeda*, fertilizer addition was recently reported to reduce carbohydrate stores in current year tissues (enhanced sink activity), while overwintered tissue contained more starch (winter assimilation stimulated without corresponding C-demand; Ludovici *et al.* 2002).

During 1999 transparent, ventilated branch bags were used to enrich small fractions of the canopy with CO_2 . Leaves of all six species responded with increased NSC concentrations, with the effect being highly significant in four of the species (Fig. 7). These leaves were tied to a theoretically almost infinite sink for additional C, given that the rest of the canopy did not receive CO_2 -enrichment. In 2001 a group of these trees was exposed to whole canopy free air CO_2 -enrichment using a new technique called web-FACE, consisting of several kilometres of fine tubes (woven into the crowns) that emit computer-controlled pulses of pure CO_2 through tiny, laser punched holes (Pepin & Körner 2002). Under a 'cloud' of *c.* 520 p.p.m. CO_2 , trees maintained higher leaf NSC throughout the season, with the enrichment effect similar to what was seen with the branch bags in 1999 (Fig. 8). The effect was not confined to leaves, but was also seen in branch wood.

In summary, natural abundance of NSC in these mature deciduous trees and NSC responses to partial and full canopy CO_2 -enrichment do not suggest that growth is carbon limited at this site. A substantial fraction of the large NSC stores may still be advantageous for buffering extreme events such as destructive hail

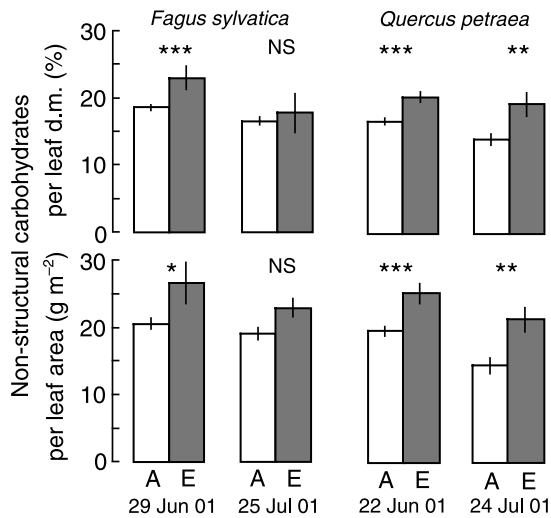


Fig. 8 NSC concentrations in leaves of 30–35 m tall deciduous forest trees exposed to full canopy CO₂-enrichment in 2001 (Ch. Körner and O. Bignucolo, unpublished data). A = ambient CO₂, E = elevated CO₂, d.m. = dry matter.

or insect outbreaks, but does not seem to be needed currently under ‘normal operation’, including masting years. The older forestry literature evidences reduced growth in years with heavy masting (Holmsgaard 1958), but more recently there is no such evidence. Deciduous trees may have to undergo almost complete defoliation for NSC to decline significantly (e.g. Reichenbacher *et al.* 1996).

MEDITERRANEAN TREES

Mediterranean sclerophylls undergo two periods adverse to growth. A cold, 4–5-month winter from November to March, and a 4–5-month drought from June to October, causing growth to be concentrated in the 2–3-month spring period, with most species flushing in April. Here I will explore the seasonal carbohydrate variation in four species in the eastern Mediterranean, where the above seasonality is very pronounced, because of the great distance from the Atlantic Ocean and the continental influence of Anatolia. NSC concentrations may be expected to be low at the end of winter and at the end of the drought period, with highs during the ‘good’ season (spring).

However, this is not what the actual NSC data collected over a 3-year period show, nor what histochemical analysis had revealed earlier (Larcher & Thomaser-Thin 1988; Larcher 2000; Fig. 9). All four species examined show an all season maximum NSC concentration in leaves and branch wood at the end of winter (March), and a significant reduction during spring. A second weak peak occurred in October 2000, at the end of a decadal drought period, with water shortage so severe that 80-year-old trees on drained outcrops died and traditional village water sources ran dry. This late summer NSC peak was not seen in

branches in the less dry September/October 1998/1999, but leaf tissue showed a repeated September/October peak also in these years in three of the four species (see bottom of Fig. 9).

The data suggest that neither developmentally quiescent periods nor periods too cold for growth are periods in which C-acquisition is limited, leading to a surplus at the end of winter and the high levels in leaves in autumn before the winter rains started. A severe drought seems to constrain both growth and photosynthesis, so that wood NSC remains low, but the increase in leaf NSC indicates a lesser depression of photosynthesis than structural growth. Leaves seem to channel energy away from CO₂-fixation during these periods of low sink activity, as indicated by fluorescence signals and photosynthesis data (in fact, more so during the cold than the dry period; Karavatas & Manetas 1999; Larcher 2000). It seems unlikely that during these two adverse periods greater availability of CO₂ could have stimulated growth. The only period with a pronounced NSC decline is spring, when sink activity is high, but NSC never became depleted and concentrations are not significantly different between July and December in three of the four species. The spring decline was sharper in species with a short flushing period (*Arbutus*, *Quercus*) than in those with a prolonged spring growth (wild *Olea*, *Pistacia*). Similar quantitative trends in mobile C-pools have been reported for leaves by Meleti-Christou *et al.* (1994) for the same four species, for *Quercus ilex* by Cherbuy *et al.* (2001) and for *Olea* by Drossopoulos & Niavis (1988). According to Cruz & Moreno (2001) starch concentrations in stems, lignotubers and roots of *Erica australis* in central Spain also decreased during spring, when plants were actively growing and reproducing.

Under CO₂-enrichment around natural CO₂-springs in central Italy, leaves of the deciduous *Quercus pubescens* contained twice as much NSC inside as compared with outside the vent area, whereas evergreen *Q. ilex* leaves showed no significant difference (Fig. 10). NSC levels in branch wood paralleled leaf values. These trees grew for their whole life under elevated CO₂. Tree-ring data suggest that during initial regrowth in open stands, CO₂ significantly stimulated growth of *Q. ilex* in two of such spring areas (Hättenschwiler *et al.* 1997a). The stimulation was relatively larger in dry years, perhaps due to greater benefits from soil water savings due to CO₂-induced reductions of stomatal conductance during the critical part of the year (Tognetti *et al.* 1998). However, after *c.* 25 years, when the canopy became closed, the growth stimulation vanished. The adjustment was largely driven by slow morphological changes in the canopy (drastically reduced branching and leaf area per given branch age, Fig. 11). Strong initial CO₂ effects on growth are reported from post-fire regrowth oak-scrub in Florida (Hungate *et al.* 1999), perhaps reflecting favourable conditions for growth, similar to the early regrowth situation in *Quercus ilex* described by Hättenschwiler *et al.* (1997a).

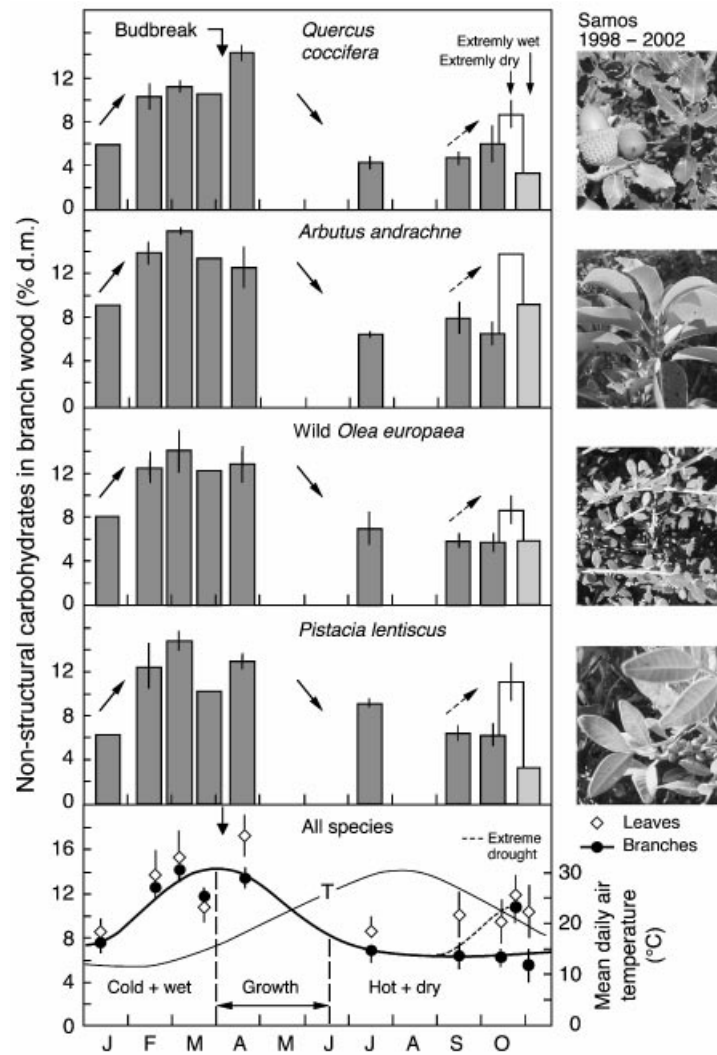


Fig. 9 The seasonal variation of NSC in branch wood in Mediterranean sclerophylls on the island of Samos, Greece. Data for leaves are included in the summarizing plot at the bottom (open symbols). Arrows indicate the major direction of the seasonal change. d.m. = dry matter.

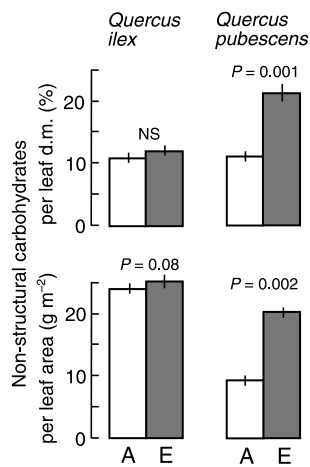


Fig. 10 Leaf NSC concentration in naturally CO₂-enriched trees around CO₂ springs in central Italy. A = ambient CO₂, E = elevated CO₂ (Körner & Miglietta 1994). d.m. = dry matter.

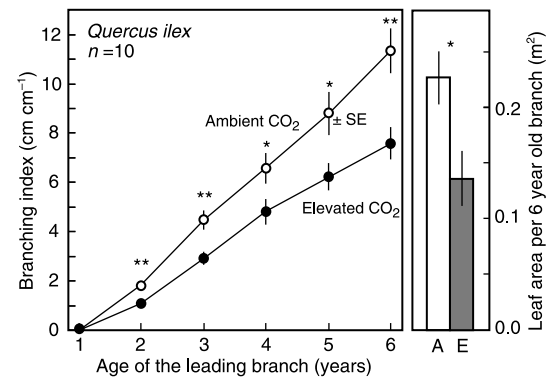


Fig. 11 In elevated CO₂ around natural CO₂-vents diminishing growth responses in Mediterranean evergreen oak are associated with morphological adjustments as trees get older (Hättenschwiler *et al.* 1997b). The branching index is defined as the cumulative length of lateral branches divided by the length of the leading branch. A = ambient CO₂, E = elevated CO₂.

In summary, woody species in such three-season climates seem to be capable of consuming more carbon for structural growth during their early growth stages and during spring, when sinks are very active, but possibly not during the rest of the year and – according to the CO₂-spring data – not once canopies become closed and trees become mature (Tognetti *et al.* 2000). Whenever environmental conditions become adverse in the Mediterranean, structural growth is affected as much, if not more than, the uptake of CO₂ and the formation of non-structural C-pools. This was evident during the cold period (Larcher 2000), and is well-known for periods of moisture shortage in general, which always affect structural growth earlier and far more significantly than CO₂ assimilation (e.g. Hsiao & Acevedo 1974; Frensch 1997). Hence, unless plants profit from CO₂-induced reductions of water consumption, drought effects should constrain C-sinks more than C-sources.

TROPICAL TREES

The last set of examples is from the seasonal tropical climate of central Panama. Most trees in the forests around Panama City are evergreen, but some shed their leaves during the so called dry season, which is not really dry, but rather shows much less rain fall than occurs during the rainy season. No doubt, this drier part of the year causes a cessation of growth in most species and visible wilting in understory vegetation, and is characterized by massive leaf litter fall and more open canopies (Wright & Cornejo 1990). Using the Smithsonian canopy crane it was possible to document the seasonal variation of NSC in all tree compartments from the top of the forest to roots, and including trunks, in 17 different tree species (Würth 1998; Würth *et al.* 1998a).

When this study was planned and conducted, we predicted a severe depletion of NSC reserves during the dry season. However, given the data for other climatic zones, it is not surprising that this prediction failed. NSC concentrations increased in all plant compartments during the drier part of the year (means for the nine best replicated species shown in Fig. 12), and this increase was largely due to starch, with sugars affected only little. Hence the NSC enrichment was not associated with osmotic adjustment, but reflected a true carbon surplus. Once again, this was not invested into structural growth, possibly because growth was more severely and directly limited by the dry conditions or by inherent tree phenology than was photosynthesis. During the rainy season, NSC pools were moderately reduced, which coincided with resumed tree growth and new leaf production. Surprisingly, variations in NSC concentrations were not or only loosely associated with the different tree reproductive phenologies, but rather mirrored moisture availability, as was also found for a subset of these species by Newell *et al.* (2002). In a way, all these findings repeat the observations

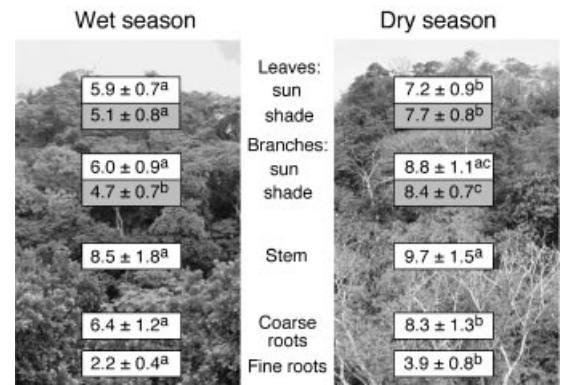


Fig. 12 The mean concentration of NSC (% dry matter) in codominant tree species of a tropical forest in Panama during the rainy and the dry season. The means are for those nine out of 17 species for which data for all organs and all dates were available, but the remaining eight species fit this overall pattern as well. ‘Sun’ refers to the top of the canopy, ‘shade’ refers to the lower part of the crowns, all accessed by a canopy crane. Different letters indicate statistically significant differences between tissue types (Würth 1998; Würth *et al.* 1998a).

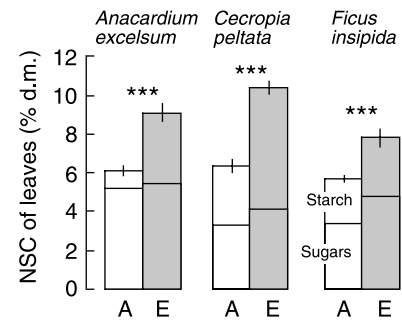


Fig. 13 The effect of CO₂ enrichment of leaves on leaf NSC in the canopy of a tropical forest (modified after Würth *et al.* 1998a). The CO₂ effect is highly significant across seasons. A = ambient, E = elevated CO₂. d.m. = dry matter.

from the temperate zone, where mass fruiting did not significantly affect tree NSC stocks.

Given the continuous high NSC concentrations in all plant parts, the trunk in particular, it looks as if trees could have used those stores, had growth activity demanded more C. There may be inherent constraints to further reduce NSC-pools associated with insurance against the risk of extreme events. However, the total forest NSC pool during the period of lowest concentrations would still suffice to completely replace the whole leaf canopy, and exceeds 10 t of NSC per ha (Würth 1998).

The effect of CO₂ enrichment on leaf NSC was studied in these trees, by pumping CO₂-enriched air emerging from the forest floor (captured under a plastic sheet) to leaves at 30–35 m above the ground (Körner & Würth 1996). Leaf NSC increased significantly in all species tested and in both dry and rainy seasons (Fig. 13). Effects were significant at the end of the day as well as after the 12-h tropical night. As soil-derived

CO₂ carries a strong stable carbon isotope signal, it was possible to identify the CO₂-enrichment signal imprinted into the leaf tissue by mass spectrometry. Lovelock *et al.* (1999) used CO₂-enriched branch bags in the same forest, and found a stimulation of photosynthesis but no effect on shoot growth or leaf NSC, but branch sapwood became significantly NSC enriched. This is the only data for mature tropical trees.

Communities of *Ficus insipida* and *Virola surinamensis*, an early and a late successional species at the Panama site, showed no growth stimulation under doubled CO₂ when grown on natural, infertile soil in open top chambers at the forest edge (Winter *et al.* 2000). All other CO₂-enrichment data are for understorey plants or seedlings/saplings grown in enclosures on fertile soils and/or in isolation, which always permitted faster growth in elevated CO₂, but such data are hard to interpret with respect to forest responses. Remarkably, growth was also found to be stimulated *in situ* (or on natural soil) when plants grew in deep shade, which reflects improved light use efficiency (e.g. Winter & Virgo 1998; Würth *et al.* 1998b) and is likely to favour climbers, which may become more aggressive and enhance forest dynamics (Granados & Körner 2002). Deep shade seems to be the prime situation under which carbon limitation plays a significant role under current CO₂ concentrations.

In summary, the data available for mature tropical trees suggest that periods of constrained growth are periods of non-structural carbon accumulation. The very limited evidence from partial CO₂-enrichment experiments in the canopy indicates a further accumulation of non-structural C-compounds under elevated CO₂, i.e. no drainage of the extra C-compounds by the whole tree. In other words, C-sink demand is not such that extra C is downloaded from places of overabundance, or translocation does not work. The fact that C-accumulation was seen in the wood of CO₂-enriched branches and not just in leaves indicates that this NSC-response was not due to a failure of phloem loading in leaves (Körner *et al.* 1995). It simply points to the inability of those trees to use additionally accumulated mobile C-compounds.

NSC responses across climatic zones: attempt at a synthesis

This survey of the mobile carbohydrate charging of mature trees across a suite of very different climates revealed several important messages:

- Whenever growth (sink activity) of dominant trees is limited by environmental or developmental constraints, we observe over-shooting source activity. Hence, these constraints appear to act more strongly upon tissue formation than on the provision of the raw material, i.e. carbon acquisition by photosynthesis.
- The seasonal variation in mobile carbohydrate concentrations is relatively small and not or only weakly associated with reproductive phenology, to the extent

that even heavy fruit loads (masting) do not affect these mobile C-pools. An exception is the NSC reduction in branch wood (not in leaves) during the spring flush of Mediterranean sclerophylls, a drop, with low concentrations sustained for much of the subsequent drought period.

- If there is a variation in mobile carbohydrate concentrations in response to low temperature or drought, the major part of it is in starch, and it is not therefore related to osmotic needs.
- Providing trees with higher CO₂-concentrations commonly enhances non-structural carbohydrate concentrations not only in leaves, but also in woody tissue, and this happens irrespective of whether only part of the canopy or the whole canopy is CO₂-enriched. In other words, these additional carbon compounds are not – or only partly – drained to carbon-demanding structural sinks.
- Experimental data further suggest that situations under which CO₂-enrichment exerts sustained stimulations of structural carbon incorporation are early regrowth (at least in warm climates) and deep shade. Light limitation makes plants extremely susceptible to CO₂-enrichment and permits them to explore microhabitats inaccessible under low CO₂ concentrations. This has significant ecological consequences for those plants whose later success is strongly determined by initial shade, particularly vines (Würth *et al.* 1998b; Hättenschwiler & Körner 2000; Granados & Körner 2002).

Having studied all sorts of organs in so many trees under so many different life conditions over a total of 10 years, it is noteworthy that there was not a single case where carbon reserves were even close to depletion during periods of high demand. In a global comparison, leaves store more NSC the lower the temperature during the growing period (Fig. 14). Among broad-leaved trees, the ever-cool montane laurophyll forest of Tenerife ranks above deciduous forests of the temperate zone lowlands, which in fact experience a warmer summer. Accounting for leaf sclerophylly (which is higher in the Laurel forest and in conifers), the difference per unit tissue volume would come out even larger. The data for woody tissue are more limited, but seem to point in the same direction.

These data, collected in a 360–370 p.p.m. world between 1993 and 2002, reveal a picture of mature forest trees so well supplied with carbon compounds that extra carbon taken up under experimental CO₂ enrichment increases mobile stocks in leaf and sap wood tissue and is not fully utilized for structural growth. It seems that adding CO₂ to the current ambient concentrations enhances the disparity between source and sink capacity under most natural conditions.

The use of carbohydrates (here represented by NSC) to identify sink-source relationships has some limitations. The most obvious one is that we do not know what is 'normal' in the sense of 'under pre-industrial CO₂ concentrations'. The chemical analysis is not trivial

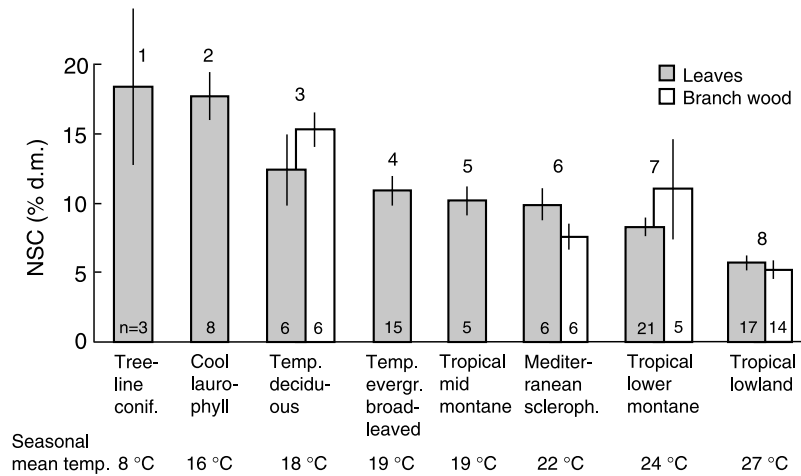


Fig. 14 A global comparison of NSC concentrations in leaves and branch wood shows a reduction with temperature during the growing season. All data were obtained with the same sampling and analysis method between 1990 and 2001. (1) Treeline conifers, see Fig. 2; (2) Tenerife, Anaga, 600–800 m; (3) Switzerland, Swiss Canopy Crane near Basel, 500 m; (4) Switzerland, Basel, 300 m; (5) Panama, Las Nubes, 2100 m; (6) Greece, Samos, 30 m; (7) Panama, Nusagandi and Cerro Azul, 400–900 m; (8) Panama, Canal Zone, 30 m. d.m. = dry matter.

and it is very difficult to compare data obtained with different methods applied to different tissues and plant species in the early part of the last century (e.g. Gäumann 1928) when ambient CO₂ concentrations were close to 300 p.p.m. Unfortunately herbarium material has usually lost most of its NSC and cannot be used as a reference. Earlier works had commonly focused on one type of tissue and one type of compound, and were often attracted by very specific compounds rather than an exhaustive representation of all or the most important compounds.

When interpreting NSC data, leaves and other tissues need to be considered as separate categories (Fig. 1). Under elevated CO₂, NSC changes in leaves are not necessarily as good an indicator of source–sink relationships as those in stems and roots. NSC concentrations in leaves (in essence starch in chloroplasts) go up under elevated CO₂ irrespective of growth conditions and seem to be tied to CO₂ concentration in a way that is not fully understood. Even in deep shade, with obvious shortages in carbon assimilates, NSC becomes increased in leaves exposed to elevated CO₂ (Körner & Arnone 1992; Würth *et al.* 1998b). Very high nutrient supply and fast growth rates do not prevent CO₂-induced NSC enrichment of leaves, suggesting translocation constraints (Wong 1990), of which phloem loading may be a part (Körner *et al.* 1995). Morin *et al.* (1992) suggested that partitioning between starch (chloroplast) and sucrose (cytosol) becomes altered by high CO₂ because of diminished photorespiration. Hence, NSC in stem tissues is a more reliable measure of a tree's carbon supply status. The best measure is the whole tree's mobile C-pool, as shown in Fig. 11 or as documented for treeline conifers by Hoch *et al.* (2002) and Hoch & Körner (2003; part of the data shown in Fig. 2), who accounted for most of the possible mobile non-protein C-fractions in all tissues. Such data are not available in the earlier literature. NSC, as used here, is

a good surrogate (as shown by Hoch *et al.* 2002), but is not an exhaustive representation of potential C-reserves.

In conclusion, the data collected by our laboratory over the last 10 years, illustrate a forest world well fuelled with C-compounds and with no significant shortages even during periods of high C-demand. Part of what we have seen might reflect inherent properties selected for in periods when CO₂ concentrations were only half as high as today, which was the case only about 180 tree generations ago. Only one or two generations ago, forests grew with 30% less CO₂ than today, a situation that has prevailed for as long as data have been available (at least half a million years, but possibly much longer).

In long-lived organisms, the formation of C-reserves has also to be seen as a safety measure for extreme events, and the mere existence of a large mobile C-pool does not mean that trees are carbon saturated (e.g. Terziev *et al.* 1997; Tissue *et al.* 1997). Yet, with all the shortcomings of such indirect evidence of tree carbon relations, the assessment of mobile C-pools permits a broad coverage of biota for which we have hardly any other data. The data presented here indicate high carbon loading of forests, perhaps indicating a limited leeway for a further CO₂-driven stimulation of growth in the future. It may well be that mature forests operate close to C-saturation under present atmospheric conditions because of growth constraints not associated with photosynthesis. The 'web-FACE' technique for CO₂-enrichment (Pepin & Körner 2002), which is now available, permits testing of this possibility with reasonable effort in otherwise unaltered environmental conditions in natural, multispecies forests.

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