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Leaf carbohydrate responses to CO_2 enrichment at the top of a tropical forest

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Abstract The accumulation of non-structural leaf carbohydrates is one of the most consistent plant responses to elevated CO_2. It has been found in both fast-and slow-growing plants and is largely independent of the duration of exposure. Changes in leaf quality are thus to be expected, irrespective of other plant responses to atmospheric CO_2 enrichment. However, there is no experimental evidence from tropical forests, the biome with the largest biomass carbon pool. Here we report in situ mesophyll responses of mature tropical trees to a doubling of CO_2. Individually CO_2-enriched leaves on 25 to 35-m-tall forest trees living at 26–35°C can be assumed to experience little sink limitation, and so, may be expected to exhibit no or very little carbohydrate accumulation. We tested this hypothesis using the leaf cup method on leaves accessible via the canopy crane of the Smithsonian Tropical Research Institute in a semi-deciduous tropical forest in Panamá. We also investigated the influence of the leaf-specific light regime, another possible environmental determinant of leaf carbon gain and mobile leaf carbohydrates. Total non-structural carbohydrates (TNC) reached a new steady state concentration after less than 4 days of exposure to twice ambient CO_2 concentration. Against expectation, all four tree species investigated (*Anacardium excelsum*, *Cecropia longipes*, *C. peltata*, *Ficus insipida*) accumulated significant amounts of TNC (+41 to +61%) under elevated CO_2. The effect was stronger at the end of the daylight period (except for *Ficus*), but was still significant in all four species at the end of the dark period. In contrast, neither artificial nor natural shading affected leaf TNC. Taken together, these observations suggest that TNC accumulation reflects a mesophyll-bound tissue response specific to elevated CO_2, presumably unrelated to sink limitations. Thus, leaves of tropical forests seem not to be an exception, and will most likely contain more non-structural carbohydrates in a CO_2-rich world.

Key words: *Anacardium* · *Cecropia* · *Ficus* · Elevated CO_2 · Light climate

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Introduction

Tropical forests, like all other vegetation on earth, existed at atmospheric CO_2 concentrations as low as about 185 $\mu l \cdot l^{-1}$ only 20 000 years ago (Neftel et al. 1988). Plants currently experience almost twice that concentration and research world-wide is devoted to studying the potential effects of the quadrupling of atmospheric CO_2 which is expected to occur by the end of the next century. More than 80% of the global biomass is bound in forests, roughly half of which are in the tropics and subtropics (Brown and Lugo 1982). Although these forests play a key role in the global carbon cycle (Wilsey 1996; Körner 1998), their response to atmospheric CO_2 enrichment is unknown. Testing tropical tree responses to elevated CO_2 is certainly a research priority. However, in situ experimental constraints are overwhelming.

Several authors have warned against the use of seedling or sapling data to predict forest responses (Eamus and Jarvis 1989; Loehle 1995; Körner 1995; Hättenschwiler et al. 1997). However, experiments with whole, natural forest canopies are not yet practical, leaving a critical gap in our understanding. We apply a technique which allows us to simulate CO_2 enrichment in small fractions of a mature forest canopy. Although work with parts of crowns is not a substitute for whole-canopy studies, some questions can still be approached with greater confidence than if seedlings or young, vigorous plantations of trees are examined. One of these questions is whether mesophyll cells in the canopy

accumulate non-structural carbohydrate even when carbon sink size is potentially very large (a whole tree *versus* few leaves).

A great number of studies have documented that total non-structural carbohydrate (TNC) concentration increases under elevated CO_2 and that this response may even persist whilst other responses may eventually disappear (Körner and Miglietta 1994). As was discussed in the latter paper, the important point is that TNC accumulation was observed irrespective of growth conditions (except under extreme nutrient starvation). Hence, it was supposed that the increase in starch and/or sugars in leaves experiencing high CO_2 resulted from direct mesophyll responses and was not, or was only partially, related to the activity of carbon sinks. One of these mesophyll-bound causes for TNC accumulation may be phloem loading, where symplast loaders (dominant in the tropics) are at a disadvantage compared to apoplast loaders (Körner et al. 1995). However, high temperatures in the tropics should largely eliminate such transport constraints (Van Bel 1989), hence other explanations need to be explored.

TNC responses to high CO_2 in tropical plants have so far been studied in the greenhouse and in the deep shade of the tropical forest understory. In model communities in relatively fertile soil (Körner and Arnone 1992), a massive accumulation of leaf TNC was found in all 15 species and all canopy strata tested. In a second experiment of similar design, in which nutrients were maintained at the lowest possible level that permitted plant growth and survival, no such effect was seen (Arnone et al. 1995). It seems almost certain that the massive and general increase in starch that was observed in the first experiment, was co-determined by greenhouse night temperatures as low as 20°C, which probably restricted phloem loading and diminished respiratory losses despite truly tropical daytime temperatures.

A recent in situ CO_2 enrichment study of understory plants in Panama found TNC stimulation by elevated CO_2 even when light levels remained below 1% of full sunlight (Würth et al. 1998). The same was observed at the bottom of the dense canopies tested by Körner and Arnone (1992). These observations lead us to consider the possibility of a TNC response which occurs independently of source-sink relationships. To further explore this possibility in the same context, we also used the canopy crane to test TNC responses to leaf shading (ambient CO_2 only) in two of the species receiving the CO_2 treatment. We also compared natural TNC abundance in sun and shade leaves within the upper part of the forest canopy. If TNC levels in the mesophyll reflect a leaf's carbon balance (e.g. oversupply or shortage of C), then a persistent reduction in quantum supply should reduce TNC.

The rationale of including a completely different environmental driver of photosynthesis to test TNC responsiveness rests on the assumption that TNC should also vary in proportion to this variable if TNC concentrations are reflecting source-sink relationships (in the case of shade a reduction of source activity; cf. Morin et al. 1992). If this were not the case, as Morin et al. (1992) found in clover, we could conclude that the response is CO_2-specific (as these authors suggested).

The combination of these manipulative and comparative tests should permit us to test the following scenarios:

1. Neither CO_2 enrichment nor shading influence leaf TNC in any way. This would mean that leaf TNC is carbon balance independent (autonomous internal setting of TNC).

2. Only shading affects (reduces) TNC. This would indicate that TNC is sensitive to the carbon supply/demand ratio (response to light environment), but CO_2 enrichment either does not induce any alteration of the source/sink balance (full photosynthetic downward adjustment, CO_2 saturation) or is accompanied by full dissipation of any extra assimilates above a "set" threshold-TNC level.

3. Only CO_2 affects (increases) TNC. This would also indicate that TNC is sensitive to the source/sink ratio in the leaf, but only above a certain TNC concentration (a minimum leaf TNC level), and that reduced light does not diminish this threshold value.

4. CO_2 enrichment raises and shading reduces TNC. This would suggest a general carbon balance link to leaf TNC (no threshold effect over the ranges of CO_2 and light considered) and a potential for enhanced carbon assimilation at elevated CO_2.

Although this list of possible interpretations is not exhaustive, these appear to be the four most plausible lines of reasoning. Scenarios 2 or 3 would be the most difficult results to explain.

The current project was designed to test these scenarios in leaves, which were most likely unlimited by sink size, since the rest of the canopy received no CO_2 enrichment and tropical temperatures could be assumed to minimize any carbohydrate transport constraints. Hence, the TNC responses observed here, if any, should be smaller than those that might be expected if the whole canopy were CO_2-enriched.

Materials and methods

Site description

The experiment was conducted in a secondary semi-deciduous tropical forest at the crane site of the Smithsonian Tropical Research Institute at the Parque Natural Metropolitano (8°58′N, 79°34′W) near Panama City, Republic of Panama. Annual precipitation averages 1740 mm, the dry season lasts from late December to early May, and the annual mean temperature is 27°C (Kitajima et al. 1997). The canopy leaf area index (above the understory) was about 4 during the wet season in 1993 (plant canopy analyzer, LAI-2000, LI-COR Inc., Lincoln, Neb. USA; the reference reading was taken above the canopy immediately before readings were taken below the canopy).

Experimental plants

For the manipulative experiments with CO_2, we selected four tree species commonly found in Panama. *Anacardium excelsum* (Barter and Blab.) Skills is characteristic for Panama and is known from Costa Rica to Ecuador and from Venezuela. *Cecropia longipes* Pitt. is a gap species of the tropical moist forest which is only known from Panama. *C. peltata* L. is found in Panama as a characteristic species of tropical and premontane moist forest and inhabits old tree fall areas from Mexico to Columbia. *Ficus insipida* Willd. is a gap species that is frequently found in the tropical moist forest of Panama ranging from Mexico to Brazil (Croat 1978).

Ficus and the *Cecropia* species are early successional whereas *Anacardium* is an intermediate species between early and late successional. We subsequently refer to species by genus except for *Cecropia*. All individuals are 25–35 m tall and mature (reproductive) trees. Treatments were applied to fully sunlit leaves of two individuals per species, except for *C. peltata*, for which only one individual was available in the crane area. Seventeen additional tree species were sampled for the sunlit-shaded leaf comparison in the canopy.

CO_2 enrichment

We mounted small transparent cups (clear plastic single serving jelly containers, 35 mm × 43 mm, 16 ml, 0.77 g) to the lower side of large hypostomatous leaves and flushed them with ambient air, or air containing approximately twice current ambient CO_2 concentration. The desired CO_2 concentration was produced by a simple gas mixing system (Körner and Würth 1996). CO_2-rich air (about 2000 µl l^{-1}) rising from the forest floor was collected under a sheet of black plastic tarpaulin and pumped to a gas-handling station (waterproof container tied to a major branch) in the canopy. This gas flow was mixed with ambient air to achieve a CO_2 concentration of about 700 µl l^{-1}. In the long run, actual CO_2 concentrations deviated from the set point by less than 100 µl l^{-1} (manual adjustment every 2nd day). Control cups were flushed with ambient air sampled in the leaf canopy by a second pump. The 15–20 leaf cups per treatment were supplied with gas via a charcoal filter, manifold and 6-m-long silicon tubes (inner diameter 2 mm; taped to branchlets and petioles). The cups had a 5-mm-diameter inlet and a vent on the opposing side, also used for gas sampling (spot measurements). With a non-toxic silicon adhesive (Medical adhesive B, Dow Corning, Sophia Antipolis, France) we pasted the cups to the lower side of leaves, avoiding major veins. The adhesive solvent was allowed to evaporate from the cup's collar before it was attached to the leaf. The flow rate through an individual cup was between 350–420 ml min^{-1}, which allows a maximum depletion of CO_2 concentration in ambient air due to photosynthesis within 30 and 60 µl l^{-1} with the largest differences seen in fully illuminated *Ficus* leaves. Amongst the species we tested, *Ficus* is known to have the highest photosynthetic capacity which ranged from about 10–30 µmol CO_2 m^{-2} s^{-1} (Kitajima et al. 1997; Zotz et al. 1995). Thus, averaged over a whole day, it is likely that our treatment created "ambient" TNC concentrations slightly lower than those which would occur without cups (a mean of −20 µl l^{-1} across our set of species and times of day seems realistic). This phenomenon contributes to the relative size of the CO_2-induced TNC differences seen in the evening.

The method and its performance were described in more detail by Körner and Würth (1996). They showed that micro climate artifacts due to the cups were small. Temperatures were similar inside and outside of the cups, which was a consequence of the very low radiation levels beneath the thick leaf blades. Humidity inside the cup was slightly higher than outside, because of transpiration. From porometer measurements in *Anacardium* it appeared that leaf conductance was increased under the cup irrespective of CO_2 concentration. The CO_2 enrichment effect was confined to the patch of leaf under the cup. We found no "leakage" of the treatment effect in the form of enhanced TNC or altered $^{13}C/^{12}C$ isotope ratio in the leaf tissue surrounding the cup.

Experimental design for CO_2 enrichment

Treatment and control cups were mounted on neighboring leaves, to minimize differences in ambient climatic conditions. To cover potential variation (diurnal as well as seasonal) we carried out the whole treatment procedure two to ten times per species (one individual per species at each time). After 4–7 days, when carbohydrate accumulation had reached a new steady state (determined in test runs), and depending on weather conditions (and crane availability), half of the leaves were harvested in the evening and the remaining half early the next morning. On some days we also sampled at 09 00, 12 00 and 15 00 hours, which, however, reduced the sample size at each harvest.

Experimental leaf shading

We taped 5 cm × 8 cm packages of regular white agro-fleece in several layers (5 layers = 50%, 10 layers = 75% reduction of solar radiation) to the upper side of fully sunlit mature leaves in the crowns of *Anacardium* and *Ficus*. We harvested the leaves after 5–7 days of in situ exposure to natural light conditions and separated the center of treated and untreated leaf sections. This period can be assumed to be long enough for physiological acclimation at the cell level. In addition, we sampled fully sunlit leaves and leaves from very shaded positions in the interior of the upper tree canopy for a total of 17 species (1–3 replicates per species). There is no light data available for each of these leaves, but spot measurements suggest that midday solar radiation was certainly less than 20% of that on fully sunlit leaves (mostly substantially less than 10%).

Harvest, storage and handling time of CO_2 or "shade" treated leaves

All treated leaves of one cohort were harvested within 15–30 min, from the crane. The samples were dried at 65°C for 24 h (in total four types of leaf samples for each species and sampling occasion in the CO_2 experiment, and two samples in the shading experiment). We tested the effect of sample handling time on TNC and found that TNC concentration was not significantly affected as long as handling time did not exceed 2 h.

Analysis of non-structural carbohydrates and nitrogen

The enzymatic method used requires samples to be ground and boiled for 40 min in distilled water. The soluble fraction was then treated with invertase and isomerase and analyzed for glucose using a Hexosekinase reaction kit (Sigma Diagnostics St. Louis, Mo., USA). In a second step, the insoluble material (including starch) was incubated for 20 h at 40°C with the dialysed crude enzyme Clarase (a fungal α-amylase from *Aspergillus oryzae*; Miles Laboratory Inc., Elkhart, Ind., USA). Starch and sugar standards as well as a laboratory standard of plant powder were used as controls for all analyses. Carbohydrates other than starch, sucrose, fructose and glucose are not covered by this assay (for more details see Körner and Miglietta 1994). Leaf nitrogen concentration was determined using a CHN analyzer (Model 932, LECO Instruments, St. Joseph, Mich., USA).

Statistical analyses

We analysed all data by type III ANOVA (JMP version 3.1, released by SAS Institute, Cary, N. C. USA). For CO_2 effects, tests were run for (1) CO_2 as the controlled factor with two levels, (2) harvest time, and (3) seasonality. Each species was tested separately. In addition, we tested the "equality of experimental leaves" by comparing data from the untreated leaf sections of both CO_2

treatments (i.e. tissue outside the cup-covered leaf section) by a *t*-test, and found the leaf quality to be the same for leaves of both the ambient and the elevated-CO_2 treatments. Similarly, the "cup-only effect" was tested with data for tissue from inside and outside the cups flushed with ambient air.

Results

CO_2 responses

TNC concentration increased during the daylight period in all species (a trend reversed in late afternoon only in *Ficus*) and this natural increase was enhanced under elevated CO_2 (Fig. 1). Generally, starch concentrations showed a more pronounced diurnal cycle and a greater CO_2 stimulation than sugar concentrations. The CO_2 stimulation in TNC is better illustrated (and statistically significant) in the much larger set of samples taken in the morning and evening (Fig. 2; for detailed information about sugar and starch consult Table 1; for statistics see Table 2). In *Anacardium*, for instance, sequential harvests (shown in Fig. 1) revealed no significant diurnal change in TNC, but the mean for all evening data was clearly enhanced (Fig. 2).

When data for all harvests and both seasons was pooled for each species, a significant increase of TNC concentration under elevated CO_2 was seen in the morning (+24 to +54%, $P < 0.03$) as well as in the evening (+38 to +62%, $P < 0.01$). In the morning

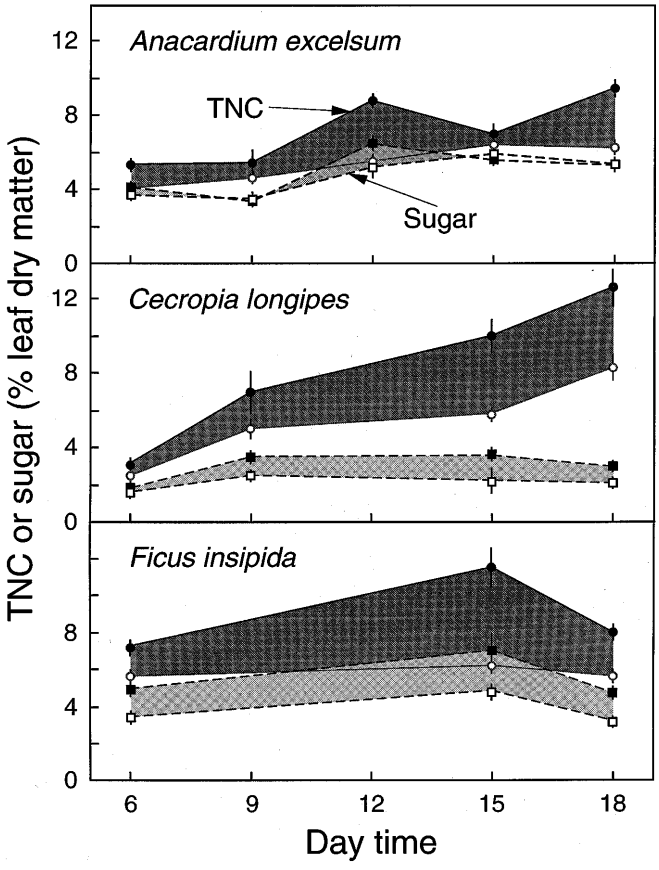

Fig. 1 Diurnal course of mean total non-structural carbohydrate (TNC) concentration in leaf tissue receiving ambient or elevated CO_2 concentration. *Error bars* indicate SEs. (*Dark symbols* elevated CO_2, *open symbols* ambient CO_2, *solid lines* TNC, *dashed lines* sugar). The *shaded area* illustrates the CO_2 effect (*dark* for TNC and *light* for sugar). Since the number of sampling days for such sequential harvests varied between 1 and 3, data were pooled irrespective of the date (4–20 leaves per CO_2 treatment and time of day)

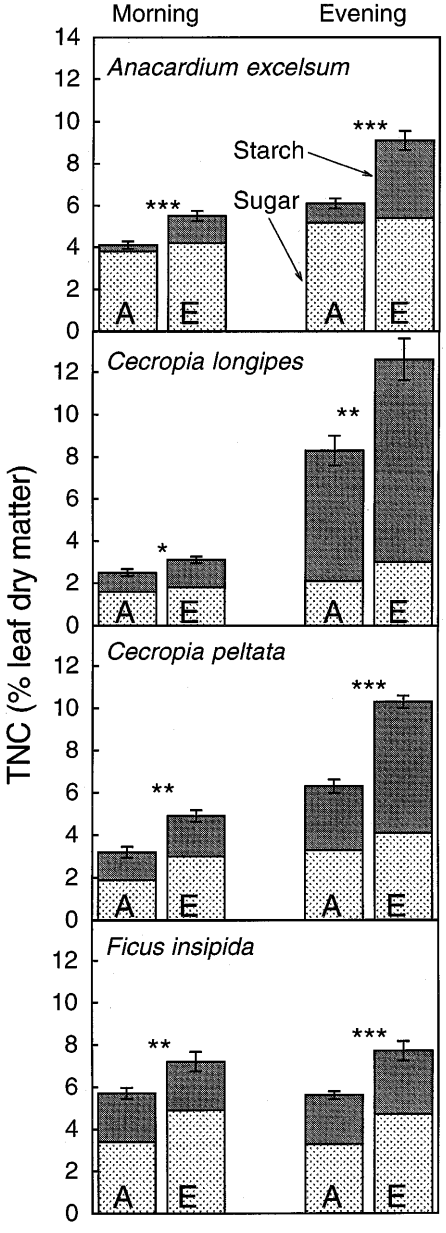

Fig. 2 The effect of in situ CO_2 treatment (*A* ambient, *E* elevated) on leaf TNC concentration and its components (sugar and starch) for all four species tested. End of day samples were collected on the evening before the morning samples were collected, thus the differences between morning and evening data reflect the remaining signal after one tropical night. Since the number of days varied between 1 and 5 (mostly 3), data were pooled irrespective of the date (n = 6–25, mostly around 15 leaves per treatment and daytime). *Error bars* show SEs for TNC only. Significant differences between ambient and elevated CO_2 are indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 1 Means, standard errors and in brackets number of leaves per treatment for the diurnal change of sugar and starch concentrations (% dry matter) for leaves grown under low and high CO_2 and all four species tested

Species	Time	Sugar				Starch			
		CO_2 low	CO_2 high	Δ Sugar (%)	*P*	CO_2 low	CO_2 high	Δ Starch (%)	*P*
Anacardium excelsum	6:00	3.8 ± 0.16 (17)	4.2 ± 0.18 (20)	+13	0.067	0.4 ± 0.14 (17)	1.2 ± 0.23 (20)	+205	0.049
	18:00	5.2 ± 0.32 (10)	5.4 ± 0.42 (9)	+04	0.685	0.9 ± 0.28 (10)	3.7 ± 0.40 (9)	+315	0.001
Cecropia longipes	6:00	1.6 ± 0.15 (5)	1.8 ± 0.12 (6)	+10	0.411	0.9 ± 0.04 (5)	1.3 ± 0.21 (6)	+48	0.094
	18:00	2.1 ± 0.09 (5)	3.0 ± 0.27 (9)	+43	0.034	6.2 ± 0.68 (5)	9.6 ± 0.80 (9)	+54	0.016
Cecropia peltata	6:00	1.9 ± 0.13 (6)	3.0 ± 0.32 (6)	+57	0.011	1.3 ± 0.22 (6)	2.0 ± 0.28 (6)	+49	0.103
	18:00	3.3 ± 0.18 (14)	4.1 ± 0.24 (14)	+24	0.014	3.0 ± 0.33 (14)	6.2 ± 0.46 (14)	+104	<0.001
Ficus insipida	6:00	3.4 ± 0.27 (17)	4.9 ± 0.34 (18)	+42	0.002	2.2 ± 0.08 (17)	2.3 ± 0.18 (18)	+5	0.586
	18:00	3.3 ± 0.31 (21)	4.7 ± 0.19 (25)	+45	<0.001	2.4 ± 0.18 (21)	3.0 ± 0.42 (25)	+28	0.184

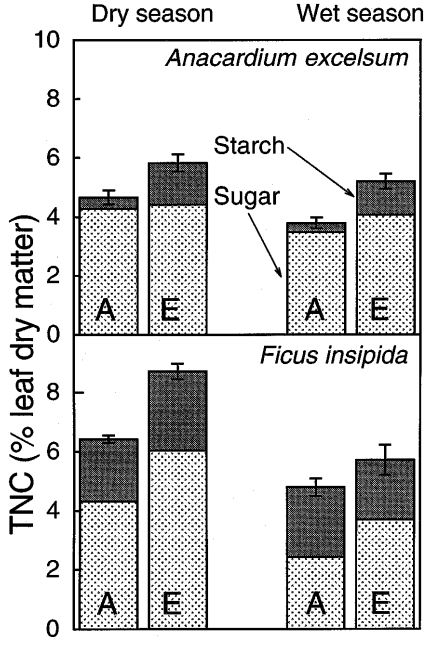

Fig. 3 Seasonal concentrations of TNC and its components (sugar and starch) in *Anacardium* and *Ficus* exposed to ambient (*A*) and elevated (*E*) CO_2 (morning data only; SE is for TNC; *Anacardium* dry season $n = 7$, wet season $n = 11$; *Ficus*, both seasons $n = 9$ leaves). In *Ficus*, dry season TNC concentration was significantly enhanced by CO_2 ($P < 0.001$), but remained unaffected in *Anacardium* ($P < 0.2$)

absolute TNC concentrations ranged from 2.5 to 5.7% at ambient CO_2, and from 3.1 to 7.2% at elevated CO_2. In the evening, concentrations were between 5.6 and 8.3% at ambient CO_2 and between 7.7 and 12.6% at elevated CO_2 (Fig. 2). Both sugar and starch concentrations contributed to this increase, with starch explaining a greater fraction of the difference, except in *Ficus* (Table 1). The CO_2 response did not change between seasons in *Anacardium*, but a greater CO_2-induced accumulation of TNC (in this case, sugar) was observed in *Ficus* in the dry season (Fig. 3).

Effect of shade

Neither TNC concentration nor its components were significantly changed by the controlled shading treatment (Fig. 4). Similarly, leaves from natural shade positions in the canopy had the same TNC concentrations as those from sunlit, top canopy positions. Looking at the composition of TNC revealed that TNC of sun leaves of a total of 17 species was composed of 56% sugar and 44% starch, compared to 59% sugar and 41% starch in the shade (Fig. 5). Hence, TNC composition did not appear to respond to quantum supply.

Leaf nitrogen concentration

Leaf nitrogen concentration on a dry matter basis was slightly reduced under elevated CO_2 in all species. No

Table 2 Results of ANOVA (type III) expressed in *P*-values of leaf TNC, sugar and starch concentrations for (a) CO_2, (b) daytime and (c) interaction CO_2 × daytime

Species	TNC			Sugar			Starch		
	a	b	c	a	b	c	a	b	c
Anacardium excelsum	< 0.001	0.012	0.012	0.06	< 0.001	0.1	0.005	0.07	0.5
Cecropia longipes	< 0.001	< 0.001	0.067	< 0.001	< 0.001	0.3	< 0.001	< 0.001	0.1
Cecropia peltata	< 0.001	< 0.001	0.004	0.001	< 0.001	0.6	0.001	< 0.001	0.6
Ficus insipida	< 0.001	0.001	0.005	< 0.001	< 0.001	0.7	< 0.001	0.2	0.002

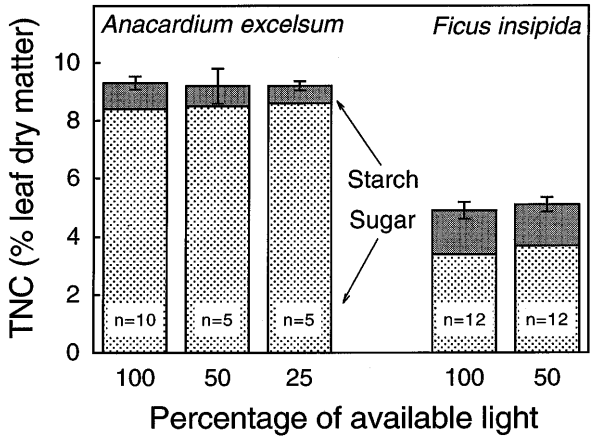

Fig. 4 Responses of TNC (and its components) to leaf shading by agro-fleece in *Anacardium* (wet season) and in *Ficus* (dry season), harvested at noon. *Error bars* show SEs for TNC

Table 3 The influence of CO_2 enrichment on leaf nitrogen concentration on a TNC free dry matter basis. Means, standard errors and in brackets number of batches of samples of 5–17 leaves collected at 2–4 dates

Species	% N (TNC free dry matter)		
	CO_2 low	CO_2 high	Δ N (%)
Anacardium excelsum	1.8 (1)	1.8 (1)	−0.8
Cecropia longipes	3.1 ± 0.07 (4)	3.2 ± 0.05 (3)	+3.0
Cecropia peltata	3.4 ± 0.10 (2)	3.5 ± 0.12 (2)	+2.4
Ficus insipida	3.4 ± 0.03 (3)	3.5 ± 0.09 (3)	+3.7
Mean (n = 4)[a]	2.9 ± 0.38	3.0 ± 0.41	+2.6

[a] Refers to number of species

significant differences were found on a TNC free dry matter basis, hence the reduction of nitrogen on a whole leaf basis was due to a dilution effect by TNC (Table 3).

Cup-only effects on TNC

In the morning cups alone had no effect on TNC concentrations (+2%, $P > 0.4$) for all four species tested. However, in the evening, when TNC reached highest concentrations, TNC concentrations were lower inside cups flushed with ambient air (−12%, $P < 0.3$ in *C. longipes*; −12%, $P < 0.08$ in *C. peltata*; −18%, $P < 0.005$ in *Anacardium*; −16%, $P < 0.001$ in *Ficus*). We assume that the same reduction occurred at elevated CO_2 and resulted in a proportional decrease in TNC in both CO_2 treatments. This is probably due to CO_2 depletion in the cups due to photosynthesis, as discussed in Methods.

Discussion

The data presented here demonstrate significant CO_2 stimulation of leaf TNC in the canopies of all four tropical tree species studied. This stimulation is largely driven by starch accumulation, but effects on sugars were also statistically significant in most cases (Table 1). A reduction in the daily dose of solar radiation to 50% or 25% of that of controls by neutral fleece filters had no effect on TNC. These leaves could acclimate physiologically to reduced quantum flux density during the treatment, but their morphology (SLA, specific leaf area) could not alter. Leaves from the natural shade within the canopy of a larger sample of species also appeared to be unresponsive with respect to TNC. Most of these leaves became shaded only after they were formed, but some of them may have a higher SLA than currently fully exposed leaves, which would cause their TNC per unit leaf area to become proportionally lower. SLA effects may also explain some of the interspecific variation seen in Fig. 5.

Overall, these findings support scenario 3 mentioned in the introduction (only CO_2 affects TNC). It appears that TNC levels at current ambient CO_2 concentrations are maintained even at sub-optimal quantum supply. The constancy of the sugar fraction in three out of four species may be associated with similar osmotic requirements, although osmotic control potentially involves a number of other compounds. Above this TNC baseline, CO_2 enrichment does indeed stimulate the build-up of greater mobile leaf carbohydrate pools under tropical field conditions, both at the end of the day and the end of the night. In the following, we will first compare these findings with literature data and then consider possible causes.

Comparison with previous observations

Absolute TNC concentrations at ambient (2.5–8.3%) and elevated (3.1–12.6%) CO_2 found here are about half of those reported for tropical plants by Körner and Arnone (1992; which is most likely due to lower night temperatures in their experiment) and those reported by Körner and Miglietta (1994) for 15 Mediterranean meadow and 2 tree species, or those for a mixture of wild

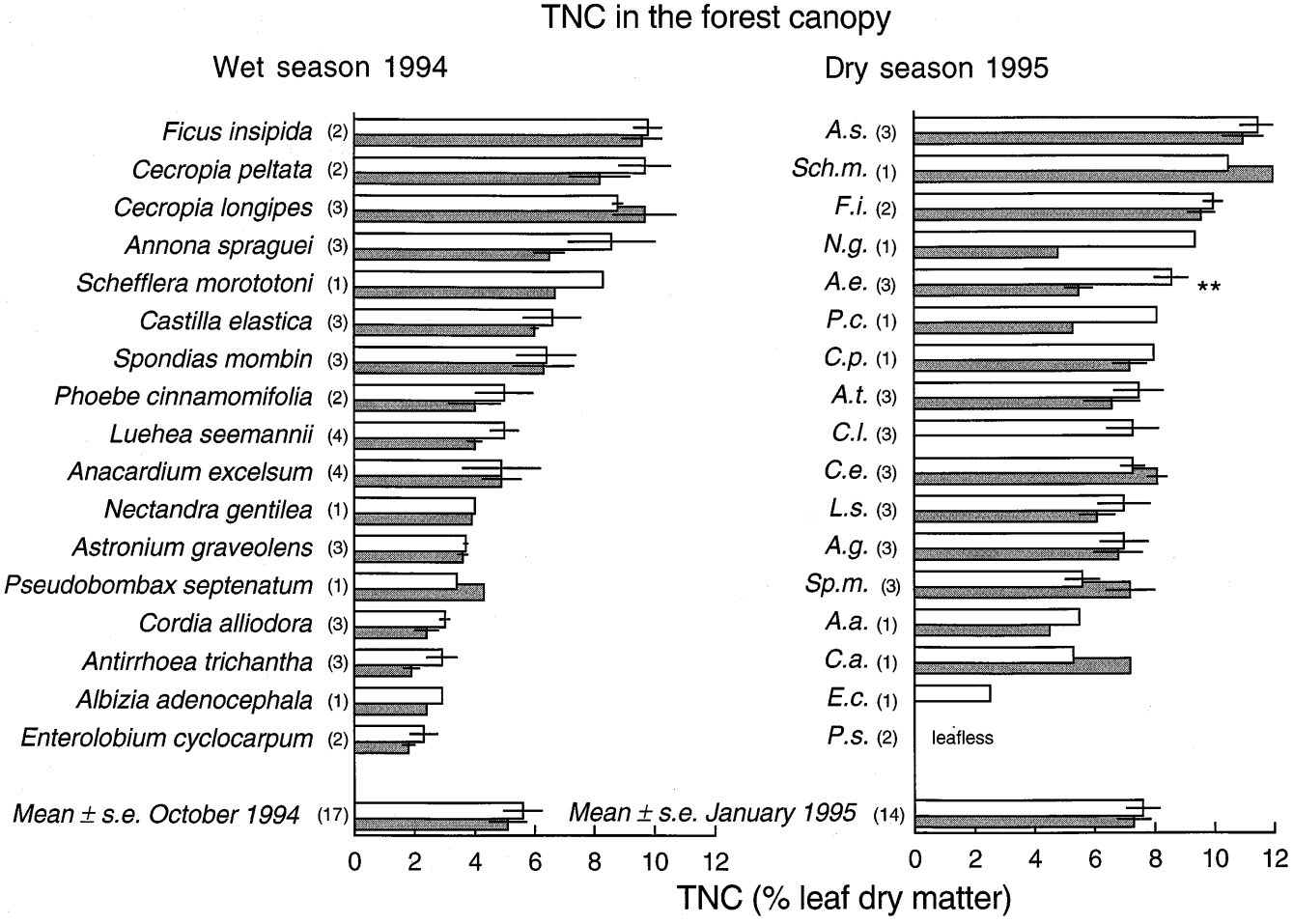

Fig. 5 The effect of natural leaf shading on TNC in the forest canopy. Sun leaves (*open bars*) were collected at the top of the canopy, shade leaves (*dark bars*) were sampled from the interior of the upper canopy from positions with less than 20% of the daily quantum supply of fully sunlit leaves. Leaves were harvested using the Smithsonian crane in the late wet season (October 1994) and in the following dry season (January 1995). *Error bars* indicate SE. For the dry season there are only data for sun leaves in *Cecropia longipes* and *Enterolobium cyclocarpum*, and *Pseudobombax septenatum* had no leaves. The means and SE per season across species are calculated only for species with complete data sets. Numbers of individuals per species and position are given in parentheses

and agricultural herbaceous plants and tree seedlings listed by Poorter et al. (1997).

The mean CO_2-induced stimulation of TNC (+42%) obtained here was of similar magnitude (though at the lower end of the range) to that observed in these earlier studies (+47% to +99%), and sugar concentrations (3–4%) were the same. Our findings of more than doubled starch concentrations under elevated CO_2 parallel those of Barton et al. (1993) for needles of mature sitka spruce trees (branch bag experiment).

Interpretation of the results

The light-shade contrasts imposed by either the designed shade or by natural shade in the canopy can be assumed to have affected photosynthesis similarly or more than the CO_2 treatment, although in opposite direction. However, these light treatments had no influence on the leaf starch pools. Following a similar rationale Morin et al. (1992) exposed *Trifolium subterraneum* to either elevated CO_2 or light, with treatments designed to permit an identical stimulation of photosynthesis by either one of these two resources. As in our study, they found contrasting responses. Enhanced starch accumulation was only observed at high compared to low CO_2 levels, not at high compared to sub-saturating light, and sucrose levels remained unaffected by both treatments. Since sink capacity was not important for the build-up of higher starch concentrations in elevated CO_2 for their nutrient-saturated plants, they suggested that high CO_2 directly affected partitioning within the mobile carbohydrate pool in cells, perhaps as a result of suppressed photorespiration. Our data suggest that a similar phenomenon occurs at well below saturating concentrations of CO_2. The high light treatment of Morin et al. (1992) was within the range that our tropical trees experienced naturally and their low-light treatment corresponded roughly to our day-long 50% fleece shading in *Anacardium* and *Ficus*.

Surprisingly, CO_2 enrichment had a strong effect on TNC even though we had enriched only a small fraction

of canopy leaves connected to an almost infinite carbon sink (a whole tree versus a few leaves), which could be expected to easily absorb the extra assimilates, unless we assume carbon saturation of trees. Leaf nitrogen data do not suggest any downward adjustment of photosynthetic capacity in leaves exposed to elevated CO_2 by withdrawal of proteins. We assume that steady state TNC accumulation in CO_2-enriched leaves was not related to an imbalance between photosynthetic capacity and transport capacity, but a more detailed examination of the cellular processes would be required to clarify this.

Stable isotope data for *Cecropia longipes* (Körner and Würth 1996) suggest a fast turnover of TNC. These authors assumed that newly synthesized TNC would carry about 50% of the soil carbon isotope signature (because of the 1:1 mixture of soil derived and ambient CO_2). However the 1.2‰ ^{13}C depletion in CO_2 fertilized leaf tissue exceeded a level which could be explained by full replacement of TNC in 1 week (0.7‰). Hence, C-compounds not included in our assay (e.g. sugar-alcohols, carbonic acids, amino acids) must have been replaced as well. Potential stomatal effects on $\delta^{13}C$ depletion can be excluded, because stomatal conductance of *Cecropia* was not reduced by elevated CO_2 (Körner and Würth 1996) – another remarkable observation.

Conclusions

The results of this field study agree with previous work suggesting that TNC accumulation under elevated CO_2 is a general phenomenon, with tropical forest trees being no exception (Körner and Arnone 1992; Körner and Miglietta 1994; Schäppi and Körner 1997). Our study further supports the idea that high temperatures result in generally diminished leaf TNC concentrations in the tropics as compared to cooler climatic zones. However in relative terms, the stimulating effect of CO_2 persists. Circumstantial evidence from several studies, including the current one, suggest that TNC accumulation under elevated CO_2 is not necessarily (or not always) linked to the activity of carbon sinks in the plants, but reflects a mesophyll-bound response (Morin et al. 1992). Irrespective of other effects of CO_2 enrichment on plants, scenarios of future ecosystem functioning need to account for higher TNC concentration and C/N ratios in green leaves. The effects seen here may be enhanced when whole trees are exposed to elevated CO_2. The complex food web in tropical forests will most probably be influenced by this physiological response of leaves to atmospheric CO_2 enrichment.

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