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In field-grown coffee trees source–sink manipulation alters photosynthetic rates, independently of carbon metabolism, via alterations in stomatal function

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Summary

• Perturbations of the source–sink balances were performed in field-grown coffee (*Coffea arabica*) trees to investigate the possible role of carbohydrates in feedback regulation of photosynthesis.

• Four treatments were applied at the whole-plant level: (i) complete defruiting and maintenance of the full leaf area, (ii) the half crop load and full leaf area, (iii) the full crop load and full leaf area and (iv) the full crop load and half leaf area. Sampling and measurements were performed twice during the phase of dry matter accumulation of fruits. Gas exchange, chlorophyll *a* fluorescence, carbon isotope labelling and steady-state metabolite measurements were assessed in source leaves.

• The average rate of net photosynthetic rate (*A*) and stomatal conductance (g_s) were larger (> 50%), and carbon isotope composition ratio was lower, in trees with a full crop load and half leaf area than in defruited trees, with individuals of the other two treatments showing intermediate values. However, differences in *A* seem unlikely to have been caused either by photochemical impairments or a direct end-product-mediated feedback down-regulation of photosynthesis.

• It is proposed that the decreased *A* in defruited coffee trees was independent of carbon metabolism and was rather directly related to a lower CO_2 availability coupled to lower g_s.

Key words: carbon metabolism, *Coffea arabica*, gas exchange, photosynthesis, source–sink manipulation, stomatal conductance.

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Introduction

The pivotal influence of sink activity on photosynthesis and carbon (C) partitioning has been well documented in several woody species, including citrus (Iglesias *et al.*, 2002; Rivas *et al.*, 2007), mango (Urban *et al.*, 2004a), coffee (Franck *et al.*, 2006) and peach (Li *et al.*, 2007; Duan *et al.*, 2008). Under conditions of decreased sink demand, end-product accumulation in source leaves is often understood to be the main factor that restricts the net photosynthetic rate (*A*; Paul & Pellny, 2003). It has been proposed that excessive starch accumulation could lead to the formation of overly large grains and could damage chloroplasts or restrict CO_2 diffusion towards the carboxylation sites (Nafziger & Koller, 1976), whereas an accumulation of soluble sugars could repress the expression of photosynthesis-related genes (Sheen, 1990). However, the sink effect on *A* appears to be somewhat contentious because a reduced sink demand is not universally

observed to cause an effect on A irrespective of whether it results in an accumulation of end products (see, for example, Lunn & Hatch, 1995; Nautiyal *et al.*, 1999). Moreover, if A is depressed by a direct feedback mechanism, photosynthesis and the activities of related key biosynthetic enzymes should decrease as end-products accumulate under decreasing source demand, but this has not always been observed (see, for example, Li *et al.*, 2007). Collectively, this conflicting information casts doubt as to whether decreased sink demand decreases A by direct feedback through carbohydrate accumulation, which is a clear factor influencing photosynthetic rates in other environmental conditions such as growth in elevated CO_2 (Stitt, 1991). As an alternative to end-product limitation, it is feasible that when A decreases as a result of weakened sink activity, partial stomatal closure, in response to changes in intercellular CO_2 concentration (C_i) (Wünsche *et al.*, 2005), may cause increased leaf temperature (T_L). If this temperature change shifts the T_L above the optimum for photosynthesis it is conceivable that it could damage the structure of photosystem II (PSII) and thereby impair A (Li *et al.*, 2007; Duan *et al.*, 2008).

In coffee (*Coffea arabica*), the activity of source photosynthetic production and sink demand appear to be highly coordinated. For example, Cannell (1971) found that when coffee trees were completely deblossomed, A decreased by *c.* 30%, whilst Vaast *et al.* (2005) showed that A was 60% lower in girdled, defruited branches than in girdled branches bearing a high crop load. Franck *et al.* (2006) noted a negative correlation between A and total soluble sugars and concluded, from sucrose-feeding experiments, that the source–sink down-regulation of A in leaves from girdled coffee branches is mediated by sucrose content in the phloem of source leaves. By contrast, DaMatta *et al.* (1997) found that starch accumulation was associated with decreases in A, whilst Ronchi *et al.* (2006), working with potted coffee displaying variant source-to-sink ratios, demonstrated that A did not correlate with starch, sucrose or hexoses but rather correlated inversely with the hexose-to-amino acid ratio. The latter finding was in accordance with the observation of Paul & Pellny (2003), that A is dependent on active pools of both C and nitrogen (N) rather than merely the carbohydrate status alone.

In the current study, source–sink imbalances were analysed via controlled defoliation and defruiting experiments, which were anticipated to modulate C assimilation and assimilate partitioning in field-grown coffee trees. We combined data of both gas exchange and chlorophyll *a* fluorescence measurements, in addition to C isotope labelling and steady-state metabolite measurements, in order to improve our understanding of the long-term effects of source–sink manipulation on photosynthesis and the process that governs photosynthetic metabolism.

Materials and Methods

Plant material and growth conditions

The experiment was carried out under field conditions with 10-yr-old trees of coffee (*C. arabica* L. 'Catuaí Vermelho IAC 44') growing as a hedgerow (east–west orientation) on a red-yellowish oxisol, in Viçosa (20°45′S, 42°15′W, 650 m above sea level), south-eastern Brazil. The site is characterized by a subtropical climate with a mean annual temperature of 19°C. It receives an average rainfall of 1200 mm, chiefly distributed from October to March. The trees were cultivated in full sunlight and were planted at a spacing of 3.0 × 1.0 m. They were trained with three to four orthotropic branches and received routine agricultural practices for commercial coffee bean production, including hoeing, fertilization and chemical control of insects and pathogens. No supplemental irrigation was provided, but there was abundant rain during the weeks preceding measurements. Twenty-four trees were selected based on their uniformity with regard to height and number of orthotropic branches.

Treatments and experimental design

The experiment was conducted during the 2005/2006 growing season. Four treatments were applied on a whole-plant level on 5 December 2005, when trees bore pinhead fruits (a stage of negligible fruit growth): (i) complete defruiting and full leaf area; (ii) half crop load and full leaf area; (iii) full crop load and full leaf area (unmanaged trees); and (iv) full crop load and half leaf area. For convenience, these treatments are hereafter referred to as T1, T2, T3 and T4, respectively. For each plant, six plagiotropic branches were tagged. These branches had 12–14 fully expanded leaves and 100–120 fruits. To improve uniformity (i.e. similar leaf area-to-fruit ratios within each treatment), the tagged branches were managed in order to bear six or 12 expanded leaves and 0, 50 or 100 fruits, according to the treatments. Unless otherwise indicated, samplings and measurements were made on cloudless days during 23–25 January 2006 and 21–23 March 2006, occasions in which the trees bore fruits in their linear phase of dry matter accumulation (assessed by evaluating fruit mass changes at 15-d intervals using an extra sample of plants). The youngest, fully expanded leaves, corresponding to the third or fourth pair from the apex of plagiotropic branches, were used.

Environment and leaf temperature

The incident photosynthetic active radiation (PAR) intercepted by leaves (in their natural angle) and the T_L were measured using a leaf chamber equipped with a PAR sensor and a fine thermocouple associated with a portable open-flow infrared gas analyser (LC*Pro*+; ADC, Hoddesdon, UK). Leaf-to-air vapour pressure deficit (δe) was calculated as described in Landsberg (1986). Other environmental data, such as rainfall,

were monitored daily from a meteorological station located *c.* 2000 m from the coffee plantation.

Growth parameters

Branch growth and leaf area were monitored at 30-d intervals during the growing season until 23 March 2006. Fruits were harvested from the mentioned tagged branches, as well as from the remaining branches, when the majority of fruits were ripe (end-May 2006). Fruits were dried according to standard agronomic practices until their humidity content reached *c.* 13%. Fruit mass and fruit fall were also assessed.

Photosynthetic measurements

Leaves were tagged for repeated measurements to be taken during the day. Natural leaf angles were maintained as far as possible during the measurements, which were conducted under ambient CO_2, light and temperature conditions. The net photosynthetic rate (A), stomatal conductance (g_s), and internal-to-ambient CO_2 concentration ratio (C_i/C_a) were measured during the day using the infrared gas analyser described under 'Environment and leaf temperature'. In addition, using a portable pulse amplitude modulation fluorometer (FMS2; Hansatech, Kings Lynn, UK), minimum (F_0) and maximum (F_M) dark-adapted (30 min) fluorescence was measured, from which the variable-to-maximum fluorescence ratio (F_V/F_M), in which $F_V = F_M - F_0$, was calculated. This ratio has been used as a measure of the potential photochemical efficiency of PSII. The photochemical quenching coefficient (q_P) and the quantum yield of PSII electron transport (Φ_{PSII}) were calculated as described in DaMatta *et al.* (2002) using a 1 s pulse of saturating light of 6000 μmol m^{-2} s^{-1}.

Biochemical analyses

Biochemical analyses (amino acids, carbohydrates (glucose, fructose, sucrose and starch), phosphorylated intermediates (glucose-1-P, glucose-6-P, fructose-6-P and inorganic phosphate (Pi)), and activities of enzymes (sucrose-phosphate synthase (SPS, EC 2.4.1.14), fructose-1,6-bisphosphatase (FBPase, EC 3.1.3.11), Rubisco (EC 4.1.1.39), ADP-glucose pyrophosphorylase (AGPase, EC 2.2.7.27), NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (NADP-GADPH, EC 1.2.1.12), acid invertase (EC 3.2.1.26), sucrose synthase (SuSy, EC 2.4.1.13) and starch phosphorylase (SPase, EC 2.4.1.1)) were assayed as previously described (Praxedes *et al.*, 2006; Ronchi *et al.*, 2006). Ribulose-1,5-bisphosphate was quantified according to Pieters *et al.* (2001). For all these analyses, leaf discs were collected after gas-exchange measurements (08:00–09:00 h, when photosynthetic rates were maximal), flash frozen in liquid nitrogen and stored at −80°C until analysed. Previous experiments revealed that both carbohydrate concentrations and enzyme activities were quite similar, irrespective of whether leaves were sampled at 08:00–09:00 h or at 13:00–14:00 h. Each replicate represented the mean of three determinations on the same sample.

Incubation of leaf discs with $^{14}CO_2$ and fractionation of ^{14}C-labelled tissue extracts

Leaves were detached from the mother plant early in the morning, re-cut under water to prevent xylemic embolism and immediately brought to the laboratory with their petioles immersed in water. This material was used for the ^{14}C-labelling pattern of carbohydrates and other cellular constituents, which was performed by illuminating leaf discs (10 mm in diameter) in a leaf chamber (LD2/2; Hansatech) under a saturating CO_2 partial pressure (approx. 5 kPa) at a PAR of 1000 μmol photons m^{-2} s^{-1} at 35°C for 30 min. Previous assays showed that incorporated radioactivity was maximal at that temperature. The CO_2 was supplied from 400 μl of 1 M $NaH^{14}CO_3$ (specific activity of 1.96 GBq $mmol^{-1}$), pH 9.3, placed on a felt mat at the base of the leaf chamber. Leaf discs were then flash frozen and stored at –80°C until required.

Frozen leaf tissues were extracted successively for 10 min at 100°C in an aqueous ethanolic series consisting of 2 ml of 80% (v/v) ethanol, 50% (v/v) ethanol, 20% (v/v) ethanol, H_2O and finally 80% (v/v) ethanol. The supernatants were combined and lyophilised. The ethanol-insoluble fraction (comprising cell-wall components, protein and starch) was separated from the soluble fraction. Components of the soluble fraction were resuspended in 2 ml of H_2O and separated into neutral (soluble sugars), anionic (organic acids) and cationic (amino acids) subfractions by ion-exchange chromatography. The incorporated radioactivity in these fractions was assessed using a liquid scintillation analyser (Beckman LS 6500; Beckman Instruments, Fullerton, CA, USA). Further details have been previously documented (Fernie *et al.*, 2001).

Other assays

Leaf tissues were oven dried for 72 h at 70°C after which total N and the stable C isotope ratio ($\delta^{13}C$) were measured, as described previously (DaMatta *et al.*, 1999, 2002).

Statistical analyses

Data were statistically examined using analysis of variance and tested for differences in significance ($P < 0.05$) using Newman–Keuls and F tests. The means presented in the Tables and Figures were obtained from six independent replications per treatment of single tree experimental plots. All statistical analyses were performed using the SAEG System version 8.0 (SAEG, 1999).

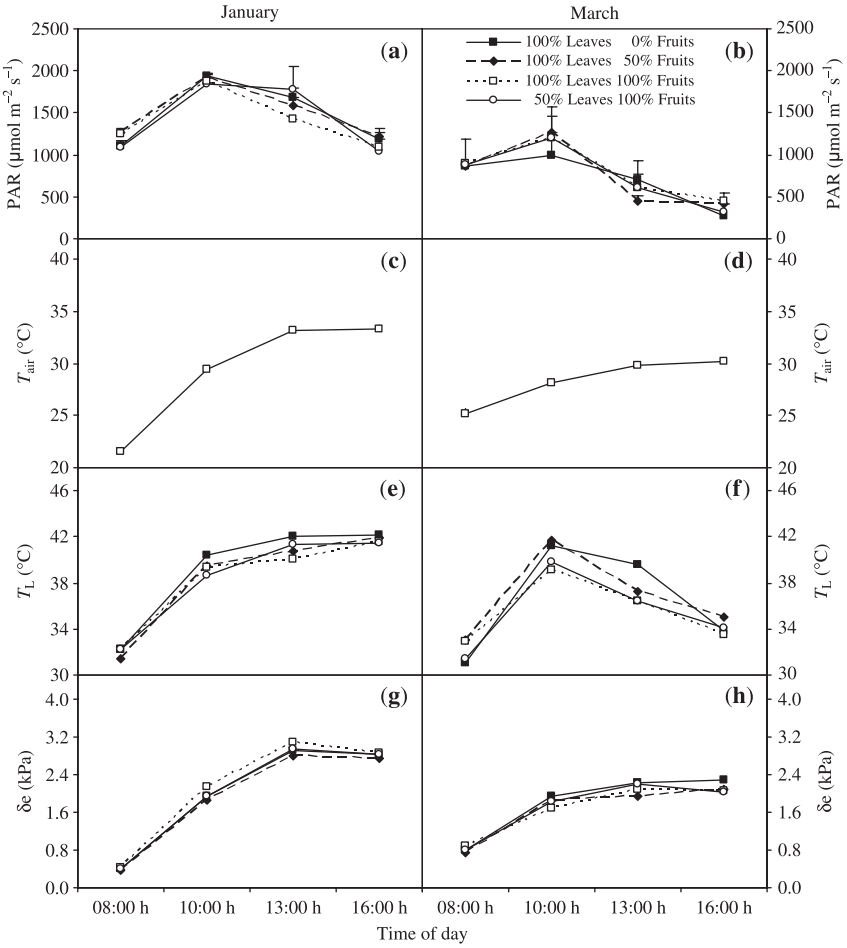

Fig. 1 The effect of source–sink imbalances performed through controlled defoliation and defruiting in field-grown coffee (*Coffea arabica*) trees on the time-course of intercepted photosynthetic active radiation (PAR) by leaves (a,b), air temperature, T_{air} (c,d), leaf temperature, T_L (e,f), and leaf-to-air vapour pressure deficit, δe (g,h). Measurements were made twice (January and March) during the linear phase of dry matter accumulation of fruits. Each point represents the mean of six replicates. Vertical bars denote the SE; when not shown, the SE was smaller than the symbols.

Results

Growth attributes are presented in Table 1. The branch growth rate decreased with decreasing source : sink ratio, although it did not differ significantly between T3 (full crop load and full leaf area) and T4 (full crop load and half leaf area) trees. For obvious reasons, the leaf : fruit ratio was lower in T4 trees and larger in T2 (half crop load and full leaf area) trees, with intermediate values in T3 individuals. The extent of fruit abscission was 13.4% in T2 trees compared with *c.* 25% both in T3 and T4 trees, whereas the mean fruit mass decreased significantly as the leaf : fruit ratio decreased. These results largely explain why the total crop yield was only 28% lower in T2 trees than in nondefruited individuals. It is worth noting that the extent of partially empty (malformed) fruits was 34% in T4 trees in comparison to 20% in T2 and T3 plants (data not shown).

The PAR intercepted by leaves under the conditions described in the present experiment was generally higher than the saturating irradiance for coffee (approx. 600 μmol photons m^{-2} s^{-1}) (Fig. 1a,b). On average, air temperature (T_{air}), T_L and δe were higher in January than in March. During the diurnal period, T_L reached values as high as, or even above, 40°C, particularly in January. At 10:00 h and 13:00 h T_L tended to be higher in T1 (complete defruiting and full leaf area) trees, especially in March when differences of up to 3°C were recorded when comparing these trees with those of the other treatments. By contrast, there were no significant differences in δe among the leaves, regardless of the applied treatments.

The overall pattern of gas exchange was characterized by low rates of A and g_s, particularly after mid-morning when both T_L and δe were particularly elevated. On several samplings, a trend for larger A and g_s with decreasing leaf area : fruit ratio was found, especially for T4 trees (Fig. 2a–d). Irrespective of treatment, A and g_s were only modestly correlated to one another other in January ($r = 0.253$, $P < 0.05$), but a stronger correlation between these variables was found in March ($r = 0.912$, $P < 0.001$; data not shown). These results suggest that part of the fluctuations of A could be explained by changes in g_s. It should be emphasized that, integrated over the course of the day, T4 trees had larger (> 50%) A and g_s values than T1 trees, with T2 and T3 individuals showing intermediate values of A and g_s (but without differing significantly) compared with T1 and T4 trees (Fig. 2a–d). Differences in both A and g_s, detected in instantaneous gas-exchange

Table 1 The effect of source–sink imbalances performed through controlled defoliation and defruiting on growth parameters in field-grown coffee (*Coffea arabica*) trees

	Treatments			
	100% Leaves	100% Leaves	100% Leaves	50% Leaves
Parameters	0% Fruits	50% Fruits	100% Fruits	100% Fruits
Branch growth (mm d^{-1})	1.21 ± 0.11^{a}	0.63 ± 0.06^{b}	0.44 ± 0.04^{c}	0.41 ± 0.04^{c}
Leaf area-to-fruit ratio (cm^2 per fruit)	–	13.4 ± 2.2^{a}	7.7 ± 1.3^{b}	4.7 ± 1.0^{c}
Total yield (g)	–	1485 ± 137^{b}	2050 ± 201^{a}	2075 ± 213^{a}
Fruit mass (g per 100 fruits)	–	65 ± 1.6^{a}	52 ± 1.1^{b}	47 ± 0.9^{c}
Fruit fall (%)	–	13.4 ± 4.2^{b}	24.6 ± 3.9^{a}	24.9 ± 3.6^{a}

Different upper case letters denote significant differences among means for each parameter ($P < 0.05$, Newman–Keuls' test). $n = 6 \pm$ SE.

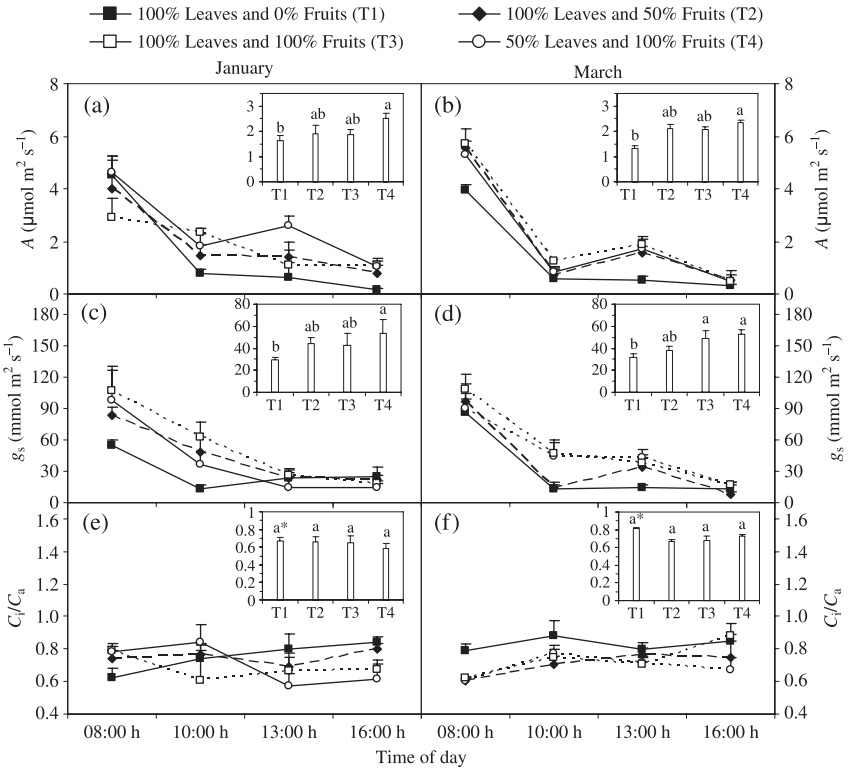

Fig. 2 The effect of source–sink imbalances performed through controlled defoliation and defruiting in field-grown coffee (*Coffea arabica*) trees on the time-course of net photosynthetic rate, *A* (a,b), stomatal conductance, g_s (c,d), and internal-to-ambient CO_2 concentration ratio, C_i/C_a (e,f). Measurements were made twice (January and March) during the linear phase of dry matter accumulation of fruits. Each point represents the mean of six replicates. Vertical bars denote SE; when not shown, the SE was smaller than the symbols. In the insets, mean diurnal values of *A*, g_s, and the C_i/C_a ratio are shown. Different letters denote significant differences among means within each time ($P < 0.05$, Newman–Keuls' test), and asterisks represent significant difference for a given parameter within sampling dates (*F* test, $P < 0.05$). $n = 6 \pm$ SE.

measurements, were also reflected in the long term: $\delta^{13}C$ was significantly more negative in T3 and T4 than in T1 trees, with T2 trees showing intermediate $\delta^{13}C$ values (Fig. 3). Some changes in C_i/C_a ratios were evident, but were not consistently associated with source–sink imbalances (Fig. 2e,f).

It should be emphasized that differences in *A* were largely unrelated to the F_V/F_M ratio, which was maintained at or above 0.8 during the day, regardless of the treatment applied. Changes in *A* were also unrelated to PSII photochemistry in light-adapted leaves because little, if any, changes were observed in Φ_{PSII} and q_P (Fig. 4).

Leaf concentrations of N, chlorophyll(*a*+*b*), glucose, fructose, sucrose, starch, glucose-6-P, glucose-1-P, fructose-6-P, Pi and ribulose-1,5-bisphosphate all remained unchanged in response to the applied treatments (Table 2). Similarly, the activities of Rubisco (total and initial activities, and activation state), SPS (maximum and selective assays, and activation state), FBPase, AGPase, SPase, invertase, SuSy, and NADP-GAPDH were also unresponsive to source–sink manipulation (Table 3). Overall, there were larger seasonal changes in these parameters than there were as a result of the experimental treatments described in this paper.

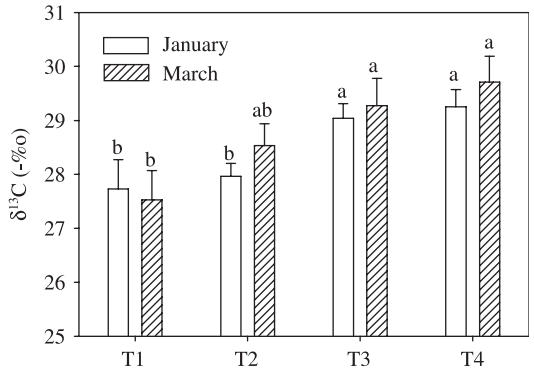

Fig. 3 The effect of source–sink imbalances performed through controlled defoliation and defruiting (T1, complete defruiting and full leaf area; T2, half crop load and full leaf area; T3, full crop load and full leaf area; and T4, full crop load and half leaf area) in field-grown coffee (*Coffea arabica*) trees on the carbon isotope composition ratio ($\delta^{13}C$). Measurements were made twice (January, open bars; and March, hatched bars) during the linear phase of dry matter accumulation of fruits. Different letters denote significant differences among means within each time ($P < 0.05$, Newman–Keuls' test). $n = 6 \pm$ SE.

The rate of $^{14}CO_2$ uptake in leaf discs isolated from the experimentally treated plants suggested that there was no change in response to source–sink imbalances (Fig. 5). Only slight changes in the partitioning of the recently fixed ^{14}C were observed between the various experimental treatments, such as that seen in the redistribution of radiolabel to amino acids. Irrespective of the experimental treatment, approx. 5% of the $^{14}CO_2$ assimilated was recovered in organic acids, 9–13% in amino acids, 80% in soluble sugars and only 1.6% in insoluble components such as starch.

Discussion

In this study, leaf area and fruit burden were manipulated in coffee trees in the absence of perturbation of other functional sinks such as roots and branches. This experimental manipulation was chosen as it probably mimics the normal growth conditions of intact coffee trees that often experience large biennial fluctuations in both vegetative and reproductive growth (DaMatta, 2004). In fact, fruits, which are by far the strongest carbohydrate sinks in coffee (Cannell, 1976), remarkably restrained the vegetative growth. For obvious reasons, competition between vegetative and reproductive organs increased with decreasing leaf area : fruit ratio, and variations in total crop load and fruit mass among fruiting treatments not only depended on the source strength but also on the sink strength of competing organs. However, for all fruiting treatments, a relatively high proportion of malformed fruits were found, even in T2 trees. Thus, it seems unlikely that fruit demand for assimilates is completely met by carbohydrates produced in source leaves. This may be a consequence of insufficient leaf area because the maximum leaf area : fruit ratio we found (13.4 cm^2 per fruit) was below

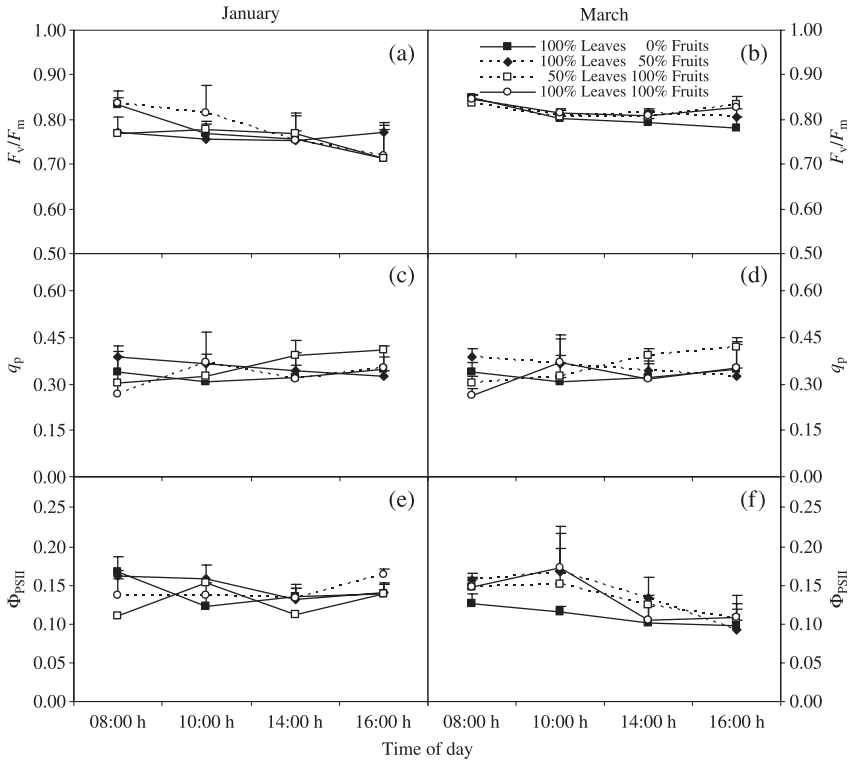

Fig. 4 The effect of source–sink imbalances performed through controlled defoliation and defruiting in field-grown coffee (*Coffea arabica*) trees on the time-course of variable to maximum chlorophyll fluorescence ratio, F_V/F_M (a,b), photochemical quenching coefficient, q_P (c,d), and quantum yield of photosystem II electron transport, Φ_{PSII} (e,f). Measurements were made twice (January and March) during the linear phase of dry matter accumulation of fruits. Each point represents the mean of six replicates. Vertical bars denote the SE; when not shown, the SE was smaller than the symbols.

Table 2 The effect of source–sink imbalances performed through controlled defoliation and defruiting in field-grown coffee (*Coffea arabica*) trees on the concentrations of nitrogen, pigments, amino acids, carbohydrates, phosphorylated intermediates and phosphate

		Treatments			
		100% Leaves	100% Leaves	100% Leaves	50% Leaves
Parameters	Time	0% Fruits	50% Fruits	100% Fruits	100% Fruits
Nitrogen (g kg^{-1} DM)	January	33.2 ± 0.8^{a}	31.4 ± 1.7^{a}	35.8 ± 0.1^{a}	31.5 ± 2.1^{a}
	March	31.8 ± 1.8^{a}	28.3 ± 1.2^{a}	27.1 ± 2.3^{a}	28.3 ± 2.3^{a}
Chlorophyll (mmol kg^{-1} FM)	January	2.39 ± 0.15^{a}	2.19 ± 0.13^{a}	1.99 ± 0.16^{a}	2.13 ± 0.26^{a}
	March	2.57 ± 0.07^{a}	2.60 ± 0.28^{a}	2.40 ± 0.13^{a}	2.04 ± 0.42^{a}
Amino acids (mmol kg^{-1} DM)	January	67.7 ± 5.4^{a}	54.2 ± 6.7^{a}	58.7 ± 3.6^{a}	$56.0 \pm 7.0^{a*}$
	March	52.7 ± 7.2^{a}	49.3 ± 5.1^{a}	42.5 ± 4.7^{a}	$38.3 \pm 5.3^{a*}$
Glucose (mmol kg^{-1} DM)	January	17.0 ± 3.0^{a}	$17.1 \pm 2.7^{a*}$	18.9 ± 2.2^{a}	24.5 ± 4.5^{a}
	March	23.2 ± 3.0^{a}	$29.6 \pm 1.8^{a*}$	21.7 ± 2.0^{a}	22.2 ± 2.7^{a}
Fructose (mmol kg^{-1} DM)	January	11.7 ± 0.9^{a}	$16.4 \pm 2.2^{a*}$	13.8 ± 1.0^{a}	13.1 ± 0.8^{a}
	March	16.7 ± 2.2^{a}	$21.4 \pm 2.1^{a*}$	15.7 ± 1.5^{a}	16.0 ± 2.0^{a}
Sucrose (mmol kg^{-1} DM)	January	137 ± 4.5^{a}	113 ± 6.4^{a}	117 ± 9.2^{a}	131 ± 8.0^{a}
	March	135 ± 11.8^{a}	114 ± 8.0^{a}	122 ± 5.1^{a}	142 ± 9.8^{a}
Starch (mmol kg^{-1} DM)	January	16.5 ± 1.1^{a}	18.5 ± 2.7^{a}	17.1 ± 3.7^{a}	$11.0 \pm 1.5^{a*}$
	March	17.2 ± 1.2^{a}	18.3 ± 2.6^{a}	22.0 ± 1.8^{a}	$17.5 \pm 0.9^{a*}$
Hexose/amino acids	January	$2.16 \pm 0.27^{a*}$	$1.82 \pm 0.25^{a*}$	1.89 ± 0.18^{a}	1.57 ± 0.21^{a}
	March	$1.45 \pm 0.25^{a*}$	$0.92 \pm 0.04^{a*}$	1.18 ± 0.16^{a}	1.04 ± 0.17^{a}
Glucose-6P (µmol kg^{-1} DM)	January	1534 ± 225^{b}	1538 ± 168^{b}	1563 ± 350^{a}	1943 ± 356^{a}
	March	1504 ± 268^{b}	1324 ± 257^{ab}	1565 ± 346^{a}	1896 ± 229^{a}
Glucose-1P (µmol kg^{-1} DM)	January	963 ± 176^{ab}	1184 ± 25^{a}	936 ± 99^{ab}	715 ± 138^{b}
	March	1352 ± 94^{a}	883 ± 66^{b}	987 ± 74^{b}	1203 ± 67^{a}
Fructose-6P (µmol kg^{-1} DM)	January	968 ± 159^{a}	1130 ± 198^{a}	1211 ± 230^{a}	1170 ± 258^{a}
	March	1023 ± 171^{a}	820 ± 83^{a}	760 ± 68^{a}	1049 ± 141^{a}
Ribulose-1,5-bisphosphate (µmol kg^{-1} DM)	January	136 ± 25^{a}	159 ± 30^{a}	200 ± 16^{a}	174 ± 16^{a}
	March	148 ± 6^{a}	138 ± 8^{a}	221 ± 22^{a}	178 ± 26^{a}
Phosphate (mmol kg^{-1} DM)	January	3.9 ± 0.46^{a}	3.1 ± 0.27^{a}	3.1 ± 0.36^{a}	3.8 ± 0.32^{a}
	March	3.7 ± 0.63^{a}	3.2 ± 0.26^{a}	2.8 ± 0.21^{a}	4.1 ± 0.55^{a}

Measurements were made twice (January and March) during the linear phase of dry matter accumulation of fruits.
Different upper case letters denote significant differences among means within each time ($P < 0.05$, Newman–Keuls' test). Asterisks represent significant difference for a given parameter within sampling dates (F test, $P < 0.05$). $n = 6 \pm$ SE.
DM, dry mass; FM, fresh mass.

the minimum presumably required (20 cm^2 per fruit; Cannell, 1976) to sustain the normal development of one fruit of coffee. At first glance, the above results largely explain the similar leaf carbohydrate concentrations, regardless of differences in leaf : fruit ratio. Even in the most contrasting treatments (T1 and T4 trees) leaf carbohydrate concentrations were quite similar, in part because the A in T1 trees was lower, but also because vegetative growth may have acted as a sink with sufficient strength to prevent carbohydrate accumulation in the leaves. From the above it follows that absolute differences in overall sink strength among treatments are narrower than previously expected. This hypothesis lends some support to explain why significant differences in A were observed only when comparing the treatments with the most divergent source : sink ratios.

The positive effect of crop load on A is consistent with previous observations for coffee (Cannell, 1976; Vaast *et al.*, 2005; Franck *et al.*, 2006). Such an effect was likely to have been mediated by an increase in g_s. Compelling evidence for this assumption is provided by the C isotope discrimination pattern and the $^{14}CO_2$ feeding studies. Larger isotope discrimination (more negative $\delta^{13}C$) can arise because of high g_s or low A (Farquhar *et al.*, 1989). Therefore, the observed decreases in $\delta^{13}C$ with decreasing leaf : fruit ratio reflect a proportionally larger increase in g_s than in A in the long term. Furthermore, as $\delta^{13}C$ reflects the internal CO_2 availability being tightly negatively correlated with the C_i/C_a ratio (Farquhar *et al.*, 1989), we propose that the long-term increases in A should have mainly occurred as a function of greater CO_2 availability associated with larger g_s. It should be noted that the contribution of potential differences in mesophyll capacity for CO_2 fixation towards the observed differences in A may be ruled out because the rate of $^{14}CO_2$ assimilation (assessed under saturating CO_2 and as such in the absence of diffusion-mediated limitations of photosynthesis) was unaffected by the imposed treatments.

Table 3 The effect of source–sink imbalances performed through controlled defoliation and defruiting in field-grown coffee trees on the activities of some enzymes associated with carbon metabolism: (Rubisco: total (V_{total}) and initial ($V_{initial}$) activities, and activation state), sucrose-phosphate synthase (SPS; maximum (V_{max}) and selective (V_{sel}) activities, and activation state), fructose-1,6-bisphosphatase (FBPase), ADP-glucose pyrophosphorylase (AGPase), starch phosphorylase (SPase), invertase (INV), sucrose synthase (SuSy) and NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

		Treatments			
		100% Leaves	100% Leaves	100% Leaves	50% Leaves
Parameters	Time	0% Fruits	50% Fruits	100% Fruits	100% Fruits
Rubisco V_{total} (μmol m^{-2} s^{-1})	January	34.5 ± 3.3[a]	34.6 ± 1.8[a]	31.4 ± 3.7[a]	37.2 ± 2.8[a]
	March	31.0 ± 3.5[a]	29.6 ± 2.6[a]	29.4 ± 3.8[a]	30.8 ± 2.3[a]
Rubisco $V_{initial}$ (μmol m^{-2} s^{-1})	January	32.5 ± 2.8[a]	28.6 ± 1.6[a]	27.7 ± 3.0[a]	27.5 ± 1.5[a]
	March	28.6 ± 3.9[a]	26.6 ± 2.8[a]	28.9 ± 2.9[a]	27.8 ± 2.1[a]
Rubisco activation (%)	January	87.3 ± 4.6[a]	83.1 ± 5.2[a]	87.9 ± 5.9[a]	76.2 ± 7.8[a]*
	March	92.8 ± 3.2[a]	89.8 ± 1.5[a]	88.8 ± 2.9[a]	92.9 ± 3.2[a]*
SPS V_{max} (mmol kg^{-1} FM min^{-1})	January	100 ± 11.5[a]	103 ± 13.7[a]	111 ± 15.8[a]	154 ± 38.3[a]
	March	94 ± 12.1[a]	88 ± 6.81[a]	95 ± 5.1[a]	110 ± 7.8[a]
SPS V_{sel} (mmol kg^{-1} FM min^{-1})	January	75 ± 12[a]	71 ± 6.9[a]	74 ± 5.0[a]	70 ± 7.9[a]
	March	76 ± 3.9[a]	74 ± 2.6[a]	82 ± 2.1[a]	81 ± 5.1[a]
SPS activation (%)	January	77 ± 3.2[a]	77 ± 12[a]	73 ± 9.6[a]	61 ± 23.5[a]
	March	84 ± 6.1[a]	85 ± 4.5[a]	88 ± 5.0[a]	82 ± 7.3[a]
FBPase (mmol kg^{-1} FM min^{-1})	January	3.51 ± 0.11[a]	3.69 ± 0.19[a]	3.32 ± 0.13[a]	3.59 ± 0.18[a]
	March	3.66 ± 0.25 [a]	3.47 ± 0.37[a]	3.65 ± 0.28[a]	3.33 ± 0.19[a]
AGPase (nmol kg^{-1} FM min^{-1})	January	60 ± 6.2[a]	47 ± 5.3[a]	46 ± 5.2[a]	54 ± 7.1[a]
	March	57 ± 4.7[a]	51 ± 3.7[a]	50 ± 9.4[a]	50 ± 13[a]
SPase (μmol kg^{-1} FM min^{-1})	January	0.21 ± 0.016[a]	0.17 ± 0.015[a]	0.19 ± 0.016[a]	0.19 ± 0.020[a]
	March	0.23 ± 0.016[a]	0.19 ± 0.012[a]	0.19 ± 0.017[a]	0.20 ± 0.020[a]
INV (μmol kg^{-1} FM min^{-1})	January	0.16 ± 0.016[a]	0.18 ± 0.024[a]	0.15 ± 0.015[a]	0.17 ± 0.029[a]
	March	0.21 ± 0.020[a]	0.22 ± 0.023[a]	0.20 ± 0.026[a]	0.19 ± 0.029[a]
SuSy (μmol kg^{-1} FM min^{-1})	January	3.05 ± 0.04[a]	2.85 ± 0.20[a]	2.72 ± 0.12[a]	2.97 ± 0.13[a]
	March	2.98 ± 0.17[a]	2.63 ± 0.24[a]	2.89 ± 0.19[a]	2.65 ± 0.29[a]
GAPDH (mmol kg^{-1} FM min^{-1})	January	63.8 ± 5.2[a]*	62.6 ± 7.5[a]	50.1 ± 7.4[a]	70.1 ± 5.8[a]
	March	40.8 ± 8.8[a]*	46.8 ± 7.3[a]	45.8 ± 9.3[a]	43.8 ± 10.9[a]

Measurements were made twice (January and March) during the linear phase of dry matter accumulation of fruits.
Different upper case letters denote significant differences among means within each time ($P < 0.05$, Newman–Keuls' test). Asterisks represent significant difference for a given parameter within sampling dates (F test, $P < 0.05$). $n = 6 \pm$ SE.
FM, fresh mass.

Differences in g_s in response to source–sink manipulation were probably unrelated to differences in C_i because in several samplings larger g_s values were not accompanied by a lower C_i/C_a ratio. Similar results have been previously reported for other species (Urban *et al.*, 2004a; Li *et al.*, 2007). In any case, increased g_s allowed increased transpiration rates (data not shown), which would largely explain the decreased T_L, as particularly found in T3 and T4 trees during the warmer periods of the day. Lower T_L, in turn, may lead to lower respiration and photorespiration rates (Lambers *et al.*, 1998), which would ultimately exacerbate the differences in A as a function of differences in leaf : fruit ratio.

Source–sink manipulations may also alter the fluorescence kinetics of chlorophyll *a*. This has previously been observed in citrus (Syvertsen *et al.*, 2003; Rivas *et al.*, 2007) and mango (Urban *et al.*, 2004b), and postulated in coffee (Franck *et al.*, 2006). In peach, Li *et al.* (2007) proposed that higher T_L, associated with low g_s and limited sink demand, impaired photosynthesis through effects on PSII reaction centres. By contrast, we have no evidence that decreased A was associated with the photoinhibition of photosynthesis because the F_V/F_M ratio during the day was near or within the optimal range (0.80–0.83 found in unstressed leaves of higher plants) reported by Björkman & Demmig (1987). In addition, differences in A were also unrelated to either q_P or Φ_{PSII}, and hence to electron transport rate.

The changes in A were rather unlikely to result from direct feedback control because the concentrations of hexose, sucrose and starch, and the hexose : amino acid ratio, were unaffected by the imposed treatments. Accordingly, the maximum extractable activities of enzymes involved in starch metabolism (AGPase and SPase), sucrose metabolism (SPS, FBPase, acid invertase and SuSy) and photosynthetic pathways (Rubisco and NADP-GAPDH), the activation state of

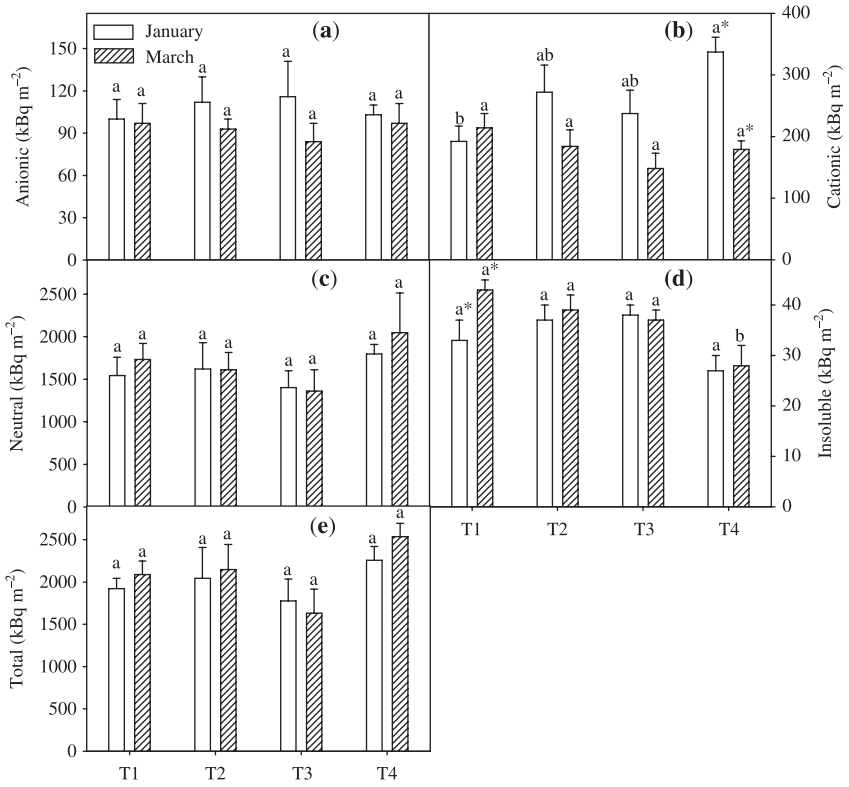

Fig. 5 The effect of source–sink imbalances performed through controlled defoliation and defruiting (T1, complete defruiting and full leaf area; T2, half crop load and full leaf area; T3, full crop load and full leaf area; and T4, full crop load and half leaf area) in field-grown coffee (*Coffea arabica*) trees on the partitioning of ^{14}C-labelled leaf tissues into anionic (organic acids) (a), cationic (amino acids) (b), neutral (soluble sugars) (c) and insoluble (mainly starch) (d) fractions, and total tissue-incorporated radioactivity (e). Note differences in scale on the vertical axes. Measurements were made twice (January, open bars; and March, hatched bars) during the linear phase of dry matter accumulation of fruits. Different letters denote significant differences among means within each time ($P < 0.05$, Newman–Keuls' test). Asterisks represent significant difference within sampling dates (F test, $P < 0.05$). $n = 6 \pm$ SE.

SPS and Rubisco, and the concentrations of hexose-P and Pi all remained unaltered, as did the actual amount and proportion of $^{14}CO_2$ diverted to the major photosynthetic fluxes. In any case, the photosynthetic apparatus clearly worked towards maintenance of a large export capacity. However, in contrast to our previous studies using young coffee plants grown in pots (Praxedes *et al.*, 2006; Ronchi *et al.*, 2006), we found, in the current study, a lower starch : sucrose ratio that was associated with a lower AGPase activity and higher FBPase and SPS activities (coupled to a higher SPS activation state), even in defruited trees. These results are consistent with the proportion of newly fixed $^{14}CO_2$ partitioned in favour of sucrose at the expense of starch. Taken together, these findings provide further support for the assumption that differences in sink strength among the treatments were relatively narrow, in addition to suggesting that changes in A in response to source–sink manipulation occurred without major changes in C metabolism.

In this study, we showed compelling evidence that the decreased A in defruited trees was largely unrelated to either photochemical impairments or direct feedback mechanisms mediated by end-product accumulation, or to a lower potential capacity of mesophyll cells to fix CO_2. Instead, the decreased A was a consequence of lower CO_2 availability for photosynthesis coupled to lower g_s. Similar results were found by Nunes-Nesi *et al.* (2007) working with transgenic tomato plants with reduction in mitochondrial activity; these plants demonstrated decreases in A that resulted from an impaired stomatal function with relatively little alteration in overall leaf metabolism, with the exception that they displayed altered starch and hexose concentrations.

In trees, decreases in g_s, associated with or without changes in C_i, with increased source : sink ratio have been reported elsewhere (e.g. Kochhar *et al.*, 2003; Zhou & Quebedeaux, 2003; Urban *et al.*, 2004a; Wünsche *et al.*, 2005; Li *et al.*, 2007; Duan *et al.*, 2008), but this response has been invariably linked to end-product accumulation. In this sense, C metabolism may thus directly or indirectly be associated with decreased g_s, and by extension A, under low-sink strength conditions (although g_s is not always tightly linked to A, as shown in tobacco with antisense decrease of Rubisco (von Caemmerer *et al.*, 2004)). By contrast, the present study, to the best of our knowledge, is the first to provide evidence that changes in g_s may take place independently of C metabolism. The exact mechanism by which the high crop load induced increases in g_s remains as yet unknown; however, it seems reasonable to anticipate that this is related to an as-yet-undefined signal associated with sink strength.

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