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Research paper

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Running head: non-structural carbohydrate dynamics in coffee

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Abstract

In coffee, fruit production on a given shoot drops after some years of high yield, triggering pruning to induce re-sprouting. The timing of pruning is a crucial farmer's decision affecting yield and labour. A reason explaining fruit production drop could be the exhaustion of resources, particularly the non-structural carbohydrates (NSC).

To test such hypothesis in a *Coffea arabica* agroforestry system, we measured the concentrations of NSC, carbon (C) and nitrogen (N) in leaves, stems, and stumps of the coffee plants, 2 and 5 years after pruning. We also compared shaded vs. full sun plants. For that purpose, both analytical reference and visible and near infrared reflectance spectroscopy (VNIRS) methods were used.

As expected, concentrations of biochemical variables linked to photosynthesis activity (N, glucose, fructose, sucrose) decreased from leaves to stems, and then to stumps. In contrast, variables linked more closely to plant structure and reserves (total C, C:N ratio, starch concentration) were higher in long-lifespan organs like stumps. Shading had little effect on most measured parameters, contrary to expectations. Concentrations of N, glucose, and fructose were higher in 2-year-old organs. Conversely, starch concentration in perennial stumps was three times higher 5 years after pruning than 2 years after pruning, despite high fruit production. Therefore, the drop in fruit production occurring after 5-6 years was not due to a lack of NSC on plant scale. Starch accumulation in perennial organs concurrently to other sinks, such as fruit growth, could be considered as a "survival" strategy, which may be a relic of the behaviour of wild coffee (tropical shade-tolerant plant).

This study confirmed that VNIRS is a promisingly rapid and cost-effective option for starch monitoring (coefficient of determination for validation, $R^2_{val} = 0.91$), whereas

predictions were less accurate for soluble sugars, probably due to their too similar spectral signature.

Keywords: Non-structural carbohydrates (NSC); visible and near infrared reflectance spectroscopy (VNIRS); perennial plant; agroforestry system; reserves.

Introduction

Coffee is a tropical perennial crop grown in approximately 80 countries. Global coffee consumption exceeds 9 million tons, of which approximately 65% is from *Coffea arabica* L. Coffee's value chain (from cultivation to marketing) is estimated to involve *ca.* 100 million people worldwide (Bunn et al. 2015).

Coffee plantations are generally established for over 30 years. Each coffee plant consists of perennial organs of which the biomass is increasing throughout the ageing of the plant (aerial stump, coarse roots) and of shoots (also called resprouts; i.e. stems which bear leaves and fruits) that are pruned regularly. Indeed, after some years, their fruit load sharply decreases and dieback of branches can occur (Muller et al. 2008, Morais et al. 2012). In Central America, especially for the dwarf varieties such as Caturra and Catimor, both phenomena are used to happening five to eight years after the last pruning. As fruit harvest usually starts only three years after pruning, a resprout is only productive for little more than half of its lifespan, a fact that affects yield, labour costs, and farm profitability directly. Any method that could help to increase total yield over the lifetime of a resprout would be of considerable benefit. One way would be to better schedule the pruning operations in order to prolong the productive span of the resprout, which would have the added benefits of sustaining the leaf-area index at the plot scale, and of providing ecosystem services such as net primary productivity (NPP) and soil protection. However, there is no proven method to date for optimizing the pruning schedule of coffee plants according to the plants' actual requirements. Such methods could be based upon physiological indicators, preferably those measurable by broadband techniques.

A common hypothesis to explain the sharp decrease in yield after 5-6 years and the possible branch dieback is that excessive fruit-sink demand for carbohydrates and nitrogen (N) could

induce resource imbalance in the old stages of the resprout, i.e., after some successive years of high yield (Cannell 1985, reviewed by DaMatta et al. 2007, Chaves et al. 2012). Available experimental evidence supports that hypothesis, showing that a high fruit production competes strongly with vegetative growth (Vaast et al. 2006, Charbonnier et al. 2017). This competition also drives the alternating production pattern, given that the number of fruits depends directly upon the number of vegetative nodes initiated during the previous year, as observed by Chaves et al. (2012), Schnabel et al. (2018) and modelled by Vezy et al. (2020). However, Chaves et al. (2012) showed no clear relationship between dieback extent and shoot or leaf non-structural carbohydrate (NSC) content.

The NSC such as starch and soluble sugars comprise reserves (particularly starch and sucrose), and reflect the balance between net photosynthetic carbon (C) uptake (source) and irreversible investments in structures or loss of C (sink; Chapin et al. 1990). Reserves build up in the plant and can be mobilized later to support biosynthesis for growth or other plant functions (Chapin et al. 1990, Richardson et al. 2013). Thus, if excessive demand by fruits is the cause of the apparent exhaustion of the coffee plants shown by reduced vegetative growth and drop in fruit production in the old stages of the resprouts, one would expect total plant NSC, particularly reserves, to be strongly depleted as well (Cannell 1985, reviewed by DaMatta et al. 2007).

Shade management in coffee plantations is likely to interfere with such NSC competition processes. The balance between photo-assimilation, fruit demand and reserves may differ between coffee plants grown in full sun (high source, large fruit sink) and in shade (lower source, but smaller fruit sink). Whether *Coffea arabica* should be grown under shade trees or in full sun remains a matter of debate. Arabica is native to Afromontane rainforest ecosystems in southwestern Ethiopia's high, cool plateaus (Gole et al. 2008). In its original habitat, wild coffee

plant grows sparsely in the deeply shaded understorey, but bears few fruits (Schmitt et al. 2010). In the field, higher yields of coffee can be achieved under full-sun conditions, but with shortened lifespan of the whole plant, faster exhaustion of the resprouts and dieback of branches (DaMatta 2004). It is generally considered that full-sun conditions enhance fruit load by encouraging a profuse blossoming that creates, subsequently, a large sink demand by the seed endosperms (Cannell 1985). Therefore, under shade, resprout exhaustion is expected to occur later and the productive span of resprouts to last longer than in full sun conditions. Moreover, shading coffee plants within agroforestry systems also improves provision of ecosystem services such as biodiversity and protection against erosion (Cerda et al. 2017).

A few investigators have already studied sugars in coffee leaves (DaMatta et al. 2008, Chaves et al. 2012, Cavatte et al. 2012, Marias et al. 2017, Avila et al. 2020), in coffee fruits (Ky et al. 2001, Bertrand et al. 2006, Vaast et al. 2006), and in other organs (Nutman 1933, Wormer and Ebagole 1965, Patel 1970). Although Frank et al. (2006) showed an apparent autonomy of fruit-bearing branches for assimilates and a limitation of photosynthesis by low fruit demand, such autonomy was less obvious in the work of Chaves et al. (2012). Moreover, DaMatta et al. (2008) and Avila et al. (2020) showed that (i) starch could accumulate at very high concentration in coffee leaves when source:sink ratio was high and (ii) the subsequent decrease in C assimilation rate was not likely mediated by variations in soluble sugar contents. However, only young plants or short-lived organs (leaves, branches) have been studied so far. Perennial stumps, in particular, have been ignored, although trunks and roots are known to be the main reserve compartments in trees (Cannell and Dewar 1994, Barbaroux and Bréda 2002, Hoch et al. 2003).

Therefore, the main hypothesis of our study was that the relationships between the apparent shoot exhaustion and resource availability in coffee resprouts would be better understood by taking into

account the perennial part of the plant (the stump) as well. As indicators of the resource balance in coffee, we selected soluble sugars – made of glucose, fructose and sucrose –, starch, total C, and total N. Glucose and fructose are assumed to be direct photosynthesis products, while sucrose is the transport form of carbohydrates and starch the main reserve compound in most trees, including coffee (Chapin et al. 1990, Cannell and Dewar 1994, Würth et al. 2004). Total C concentration was assumed to be mainly structural, whereas N concentration was undifferentiated between constitutive, soluble, and reserve N. We also considered the C:N ratio as an indicator of resource balance (Royer et al. 2013, Zhang et al. 2020). Indeed, Avila et al. (2020) showed that N metabolism was more affected than C metabolism when leaf:fruit ratio was artificially manipulated in coffee plants.

Since assessing such numerous variables via standard analytical procedures is destructive, time-consuming and expensive, future applications will require non-destructive broadband assessments. In that context, visible and near infrared reflectance (VNIR) spectroscopy (VNIRS) would offer a rapid, low-cost alternative to conventional analyses. Moreover, with such methods, samples are not destroyed and can be reused. Previous studies focusing on plant leaves and trunks showed an evident spectral signature of some particular chemical compounds, such as NSC or N, in VNIR spectral range (Curran et al. 1992, De Bei et al. 2017). In order to use VNIRS to predict a variable of interest, a calibration model (that expresses this variable as a function of the spectrum) must be firstly built on a sample dataset characterized by both VNIRS and conventional analytical methods. This calibration model needs then to be tested by cross-validation on the same sample dataset or by external validation on another dataset, for which spectral and conventional analyses have also been performed. This approach has been successfully used for characterizing the composition of aerial parts in some plants, for instance C

and N concentrations in rice (Batten et al. 1991) or wheat (Morón et al. 2007), starch content in wheat straw (Lomborg et al. 2010) and in grapevine leaves and trunks (De Bei et al. 2017), or soluble carbohydrate content in immature wheat stems (Piaskowski et al. 2016), and in whole maize plants (Campo et al. 2013). Ramirez et al. (2015) also showed the ability of near infrared reflectance spectroscopy (NIRS) to accurately predict total NSC and starch concentrations in different organs of different woody plant species. Generally, in these studies, model accuracy to predict starch and NSC concentrations was influenced by the level of variability in the dataset. Indeed, on the one hand, a sufficient variation of the variable is required to build an accurate model, but on the other hand, a sample that is too atypical in the dataset could hardly be predicted by the others (Campo et al. 2013, De Bei et al. 2017). To date, very few studies have proposed a specific prediction of each NSC compound (i.e. glucose, fructose, sucrose and starch separately) and never for different organs of the considered plant. Quentin et al. (2017) only studied predictions of each NSC in Eucalyptus leaves and Wang et al. (2016) successfully predicted sucrose and starch concentrations in rice stems only. Moreover, several studies have reported the successful use of VNIRS for predicting a range of biochemical compounds in coffee beans (Davrieux et al. 2004, Bertrand et al. 2006, Santos et al. 2012, Scholz et al. 2014), but there is no example to date of the application of VNIRS on different coffee vegetative parts with a view to documenting the plant's overall resource balance. It is thus necessary to test the ability of this method to predict NSC, C and N concentrations and C:N (chosen as indicators of plant resource balance) in different organs of coffee, to understand whether VNIRS can be used to assess coffee strategy for resource use and help optimizing the shoot pruning schedule.

The aims of this study, which was conducted at the whole-vegetative plant scale (resprouts and stumps) in a mature agroforestry system in Costa Rica, were: (i) to assess the variability of NSC

(glucose, fructose, sucrose, starch), C and N concentrations in coffee vegetative aerial organs, according to resprout age and exposure (full sun or tree shade); (ii) to understand whether NSC, and particularly reserves, are depleted by heavy fruit load, explaining subsequent collapse in fruit yield and possible resprout exhaustion; (iii) to test the suitability of VNIRS to provide new broadband tools for monitoring coffee NSC dynamics.

Materials and methods

Study site: location, soils, climate, and vegetation

The coffee agroforestry farm of Aquiares is located in the Reventazón River basin, in the Central-Caribbean region of Costa Rica, at coordinates 9°56'17"N and 83°43'41"W. It lies on the slope of the Turrialba volcano (the country's central volcanic mountain range), and drains to the Caribbean Sea. Certified as Carbon-Neutral and by the Rainforest Alliance™, it is one of Costa Rica's largest farms (6.6 km^2). The study plot was part of the "Coffee-Flux" Observatory embedded in the Aquiares farm, at the elevation of 1000 m a.s.l.

The farm's climate is tropical humid with no marked dry season and is strongly influenced by the climatic conditions in the Caribbean hillside, according to Köppen-Geiger classification (Peel et al. 2007). The mean annual rainfall at the Aquiares farm station was 3014 mm for the period 1973-2009 (Gómez-Delgado et al. 2011). The farm's soils belong to the order of Andisols according to the USDA soil taxonomy (USDA-NRCS 2005), and are developing from volcanic ejecta under weathering and mineral transformation processes. The soils have a high organic matter content and biological activity, as well as high infiltrability (Benegas et al. 2014, Kinoshita et al. 2016).

The studied plots were planted in the 1970s with variety Caturra, a dwarf mutant of Bourbon, which is subject to fruit overbearing (Wintgens 2004). The farm is managed quite intensively (upper conventional mode; fertilizing 214 kg N ha^{-1} $year^{-1}$; Standard Deviation, SD = 44 kg N ha^{-1} $year^{-1}$; 2000-2012 period). As an indicator of overall productivity, green coffee yields averaged 1,351 kg ha^{-1} $year^{-1}$ (SD = 347 kg N ha^{-1} $year^{-1}$) between 1994 and 2013. The coffee plants' leaf area index (LAI) – an indicator of vegetative vigour – varied seasonally from 2.4 to 4.4 m_{leaf}^2 m_{soil}^{-2} between 2001 and 2011 (Taugourdeau et al. 2014).

The initial planting density for coffee was 6,300 holes ha^{-1} (1.11 m between plants in the row, and 1.43 m between rows) with two stems planted per hole. Coffee aerial parts were composed of a perennial stump (*ca*. 40 cm tall, lifespan > 40 years; Defrenet et al. 2016), from the top of which emerged one or two shoots comprising a main stem (the orthotropic stem, subvertical) and several lateral branches (plagiotropic, subhorizontal), which bore leaves (lifespan ~ 0.6-2 years), flowers, fruits, and secondary branches (Figure 1). Every year, on March, approximately 15% of the coffee shoots in each plot were selectively pruned. The pruning decision was based on the farmers' observation that after a high fruit production, the shoots only emitted a small vegetative flush with a low number of leaves and of axillary nodes where new flower buds could occur. This usually occurred when the shoots were 5-y old. After pruning, new shoots, herein called 'resprouts', emerged on each pruned stump and started developing (Figure 1), and a selection was performed by the farmer to finally keep two resprouts only. Fruit yield in the studied plot increased regularly after pruning, starting very low in the first year (17.8 g per resprout in full-sun, 15.1 g per resprout under shade) to a maximum in the fifth year (170.9 g per resprout in full-sun, 140.1 g per resprout under shade; Charbonnier et al. 2017). Total resprout biomass increased in the same way, whereas wood relative growth rate (RGR), relative allocation to wood and Net

Assimilation Rate (NAR; NPP divided by leaf area) decreased in the fifth year as compared to year 3-4 (Charbonnier et al. 2017).

On the studied plots, Erythrina shade trees (*Erythrina poeppigiana* (Walp.) O.F. Cook) had a density of 7.4 trees ha^{-1}, with 15.7% canopy cover and around 20 m canopy height on average. The plot-scale maximum LAI of Erythrina was 0.67 m^2 m^{-2}, intercepting on average 14% of the annual irradiance. However, coffee plants located just below the shade tree canopy received only 30% of incident photosynthetically active radiation (PAR), and the shade tree area of influence was much larger than their crown projections (Charbonnier et al. 2013).

Sampling and experimental display

The main flowering occurred by mid-April. Harvest started in August, and was completed in January of the following year. October was the peak time of grain filling. Because starch levels in coffee leaves were reported to be highest at the time of flowering and lowest at the time of grain filling (Nutman 1933, Wormer and Ebagole 1965, Patel 1970), October 2012 was chosen for sampling, assuming that the month of October would include the moment of highest reserve depletion (i.e. the time when the resource balance would be the most critical for the plant).

Sixty different randomly selected coffee plants were sampled within a week, from 8 a.m. to 4 p.m. Indeed, Cooil (1953) reported that starch concentration in leaves remained stable over 24 h courses. Regarding exposure, half of the plants were selected from shaded locations (below the crown of a shade tree), and half from full-sun locations (> 20 m from the nearest tree). Regarding maturity, half of the plants bore 2-year-old resprouts (vegetative only), and half bore 5-year-old mature resprouts (bearing fruits). Thus, for each maturity × exposure combination, there were 15 replications of coffee plants.

Three aboveground vegetative organs (stem, leaves and stump) were sampled in each plant (Figure 1). The short-lived organs were from the resprout: (i) "stem", i.e. two opposite mid-canopy plagiotropic branches of the stem, together with their common orthotropic internode, sampled at the half-height of the orthotropic stem; and (ii) "leaves", taken without petioles from the same stem sample. The perennial organ was the stump, sampled at its mid-height, from cortex to centre, with a wood chisel. The stump wounds received healing dough. All samples were pre-stored in ice in a cool-box brought to the field, then for a few days at -20°C just before freeze-drying.

In total, 178 samples were collected: 60 leaf samples, 60 stem samples, and 58 aerial stump samples (2 were lost by accident).

Biochemical compounds assessed by reference methods

Six variables (glucose, fructose, sucrose, starch, C and N concentrations; g kg^{-1}_{DM}) were assessed in the laboratory, using reference methods described below; then, C:N ratio was computed. In the Laboratory of Biotechnology, Plants and Soil at CATIE-Costa Rica, the 178 samples were freeze-dried (VP0F freeze-dryer, Savant Instruments, Inc. Holbrook, NY, USA) and finely ground to < 0.1 mm in a ball mill. Sample powders were packed in hermetic zip plastic bags, and then shipped to Montpellier (France) for analyses. In the laboratory of CIRAD-Montpellier (Biochemical phenotyping platform), aliquots (18 to 20 mg) of each sample were oven-dried for two hours at 65°C, then assessed for glucose, fructose, sucrose and starch.

Soluble sugar and starch contents were measured according to Silpi et al. (2007) with few adjustments. The overall protocol was in accordance to that recommended by Quentin et al. (2015), with some adaptations to the plant material, as specified below. The NSC were extracted

with 1 mL ethanol at 80% for 30 min at 75°C, and then centrifuged. This protocol was repeated once with ethanol at 80%, and once with ethanol at 50%. The supernatants were pooled. Soluble sugars were contained in the supernatant, and starch was contained in the pellet. Then, 500 µL of ethanol at 80% were added on the pellet and kept at -20°C. To eliminate pigments and polyphenols from the supernatant, it was filtered through a column containing polyvinyl polypyrrolidone (PVPP) and activated charcoal prepared in 80% ethanol. After evaporation of ethanol using a vacuum concentrator (Jouan, RC 1022 & RCT 90, Saint-Herblain, France), soluble sugars were quantified by high-performance ionic chromatography (HPIC; Dionex DX-600, Sunnyvale, CA, USA) using Dionex carbopack PA1 IC column (Thermo Fisher Scientific) with pulsated amperometric detection (HPAE-PAD). The mobile phase was 0.15 M sodium hydroxide, and the flow 1 mL min^{-1}.

After evaporation of the ethanol with vacuum concentrator, starch was analysed in the pellet. It was gelatinized with 1 mL 0.02 N sodium hydroxide at 90°C for 1.5 h, then hydrolysed with α-amyloglucosidase prepared in citrate buffer pH 4.5 at 50°C for 1.5 h. Glucose produced by hydrolysis of starch was quantified as described by Boehringer (1984) using hexokinase and glucose-6-phosphate-dehydrogenase, followed by spectrophotometry of reduced nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm (spectrophotometer UV / VIS Jasco, V-530, Tokyo, Japan). All results were expressed as plant concentrations (g kg^{-1}_{DM}).

Standard errors of laboratory (SEL) for hexoses (i.e. glucose and fructose), sucrose, and starch were 5%, 6%, and 9% respectively, according to analyses of internal reference samples of the same nature (woody material) regularly carried out in CIRAD-Montpellier laboratory.

Concentrations of C and N were determined using dry combustion (CHN Fisons / Carlo Erba NA 2000, Milan, Italy) at the Eco&Sols joint research unit in Montpellier (France). Aliquots used for

these analyses were prepared by grinding them to < 0.1 mm, then oven-drying them over night at 40°C. SEL was not available for C and N contents.

Statistical analyses for reference methods

Statistical analyses were performed using the R software (R Core Team 2017) in order to study the effects of the three factors: (i) organ (leaves vs. stem vs. stump); (ii) maturity (2-year-old vs. 5-year-old shoots); (iii) sun exposure (full sun vs. under shade) on the seven studied variables. The within-group variable distributions were non-normal and heteroscedastic, and they best fitted Gamma distributions (Villaseñor and González-Estrada 2015). A group being defined by one organ / one age / one shade level. This justified the use of generalized linear models (glm) with Gamma distributions (McCullagh and Nelder 1989). Because measurements were done on organs within the same plant, individual plants were included as random variables to account for any plant effect, resulting in generalized linear mixed models (glmm) of the form 'measurement ~organ*shade*age + random(1|individual)'. A multiple comparison Tukey-Kramer test decided whether variable means between the three organs differed significantly. Secondly, maturity and exposure effects were analysed in each organ separately using glm of the form 'measurement ~ shade*age'. Because a Gamma distribution only works for strictly positive variables, three starch zero values were set to a small value below the detection limit (0.0001 g kg^{-1}_{DM}). In all tests, differences were considered significant at $P < 0.05$.

VNIR spectrum acquisition and statistical analysis

VNIR spectra were acquired at the Eco&Sols joint research unit, Montpellier (France), on finely ground (< 0.1 mm), homogeneous aliquots of *ca.* 10 g that had been oven-dried overnight at

40°C. Spectral absorbance was obtained and kept from 450 to 2500 nm at 4 nm intervals using a LabSpec 2500 portable spectrophotometer (ASD, Boulder, CO, USA).

Spectrum analysis consisted of fitting the VNIR spectra to the seven variables that had been assessed conventionally by the methods described above (glucose, fructose, sucrose, starch, C, N, and C:N ratio). Data analysis was conducted using the WinISI III-version 1.50e software (Foss NIRSystems / Infrasoft International, State College, PA, USA).

To reduce irrelevant information (e.g., resulting from light scattering), different spectral pre-processing methods were tested: no transformation (none); standard normal variate (SNV) transformation; and both SNV and de-trending (SNVD; Barnes et al. 1989). They were combined, or not, with numerical calculation of first or second derivatives. For that, three combinations of derivatives and smoothing were tested: no derivatives and no smoothing (denoted as 001); first derivatives with 4-point gap and 4-point smoothing (144); and second derivatives with 4-point gap and 4-point smoothing (244). The combination of three transformations and three derivation procedures gave nine pre-processing methods.

Principal component analysis (PCA) was then carried out to calculate the Mahalanobis distance (H) for the set of processed VNIR data as a whole, and for each organ separately (Mark and Tunnell 1985). The samples whose spectra were atypical ($H > 3$) were considered spectral outliers and omitted from further analysis. The whole set consisted of 180 samples: 59 aerial stump samples; 60 stem samples; and 61 leaf samples. The total set was divided into approximately 100-sample calibration subset and approximately 80-sample validation subset.

For each set of processed VNIR data, the calibration subset was selected by the WinISI III-version 1.50e software in order to include the most spectrally representative samples of the set. Having selected the calibration subset, a modified partial least squares (MPLS) regression was

used to develop calibration models from spectra and conventionally determined variables (Shenk and Westerhaus 1991). Six-fold cross-validation was performed on the calibration subset to determine the optimal number of terms to be included in the prediction model. The standard error of cross-validation between predicted and measured values (SECV) was then calculated. Calibration outliers (i.e. samples with residual greater than 2.5 times SECV) were removed, then another cross-validation was performed, the procedure being carried out twice. All remaining calibration samples were used to calculate the final model.

Calibration performance was assessed using the coefficient of determination (R^2_{cv}) and the ratio of standard deviation of the calibration subset to SECV (denoted as RPD_{cv}). Then, prediction accuracy was tested on the validation subset. The two performance parameters of validation were the coefficient of determination (R^2_{val}), and the standard error of prediction corrected for bias (SEPc). The ratio of standard deviation of the validation subset to SEPc, denoted as RPD_{val}, was also considered. Williams and Norris (2001) suggested that calibration models with $RPD \geq 5.0$ could be considered good (i.e. sufficiently accurate to be used in quality control), while models with $3.1 \leq RPD \leq 5.0$ are fair (sufficient for screening), and those with $2.4 \leq RPD \leq 3.0$ are poor (suitable for rough screening only).

Next, MPLS regression was carried out on each organ separately. Because of the small sample sets (≤ 60 samples per organ), only calibrations using cross-validation were carried out.

The detailed description of this section is given in supplementary material.

Results

Effects of organ, maturity, and exposure on biochemical variables assessed by reference methods.

<Insert Table 1 here>

We distinguished three groups of variables according to observed CV (Table 1): (i) glucose, fructose and starch were highly variable, with CV above 100%; (ii) sucrose, N and C:N were quite variable, with CV around 50%; whereas (iii) C was slightly variable, with CV close to nil.

The organ had a very clear effect on every biochemical variable. Within the plants, N, glucose, fructose and sucrose concentrations increased with proximity to the photosynthetic source (stumps < stems < leaves). In contrast, C, C:N and starch increased downwards. Total NSC content was higher in leaves than in stems and stumps. Maturity had a strong effect too: N concentration was lower in leaves and stems of mature (5-year-old) resprouts than in those of immature (2-year-old) resprouts. By contrast, glucose, sucrose, and noticeably total NSC and starch concentrations were higher in stumps bearing mature resprouts than in stumps bearing immature resprouts (Table 1; Figure 2d), indicating storage in perennial organs at the mature stage, despite competition exerted by fruit growth at that age. Unexpectedly, exposure had the least effect: glucose, fructose and sucrose remained steady in leaves. We also noted very little interaction between maturity and exposure.

<Insert Figure 2 here>

More details are provided below, especially for N, glucose and starch.

Nitrogen concentration averaged 17.0 ± 10.5 g kg^{-1}_{DM}, and was variable (CV = 62%). The decrease by a factor of 4 in N from the leaves (31.0 ± 2.6 g kg^{-1}_{DM}) to the stems

(12.5 ± 2.9 g kg^{-1}_{DM}) and finally to the stump (7.1 ± 1.1 g kg^{-1}_{DM}) was highly significant. In addition, a very highly significant decrease in N concentration was observed between 2- and 5-year-old resprouts: from 33.0 ± 1.8 for leaves of 2-year-old resprouts to 29.1 ± 1.7 g kg^{-1}_{DM} for leaves of 5-year-old resprouts, and from 15.1 ± 1.4 g kg^{-1}_{DM} for 2-year-old stems to 9.9 ± 1.2 g kg^{-1}_{DM} for 5-year-old stems. Exposure did not significantly affect N concentrations in any of the three organs. The maturity × exposure interaction was significant for stems, but not for leaves.

Glucose concentration averaged 4.4 ± 4.9 g kg^{-1}_{DM}, and was highly variable (CV = 110%). Very significantly, glucose was divided by a factor of 5 from the leaves (10.4 ± 3.7 g kg^{-1}_{DM}) to the stems (2.1 ± 1.0 g kg^{-1}_{DM}), and by a factor of 3 from the stems to the stumps (0.64 ± 0.21 g kg^{-1}_{DM}). In the leaves, neither maturity nor exposure had a significant effect (Figure 2a). The 2-year-old stems were significantly richer in glucose (2.5 ± 1.2 g kg^{-1}_{DM}) than the 5-year-old ones (1.8 ± 0.8 g kg^{-1}_{DM}), without exposure effect. In the stumps, the contrary was observed, with significantly more glucose in stumps bearing 5-year-old resprouts than in those bearing 2-year-old resprouts (0.7 ± 0.2 vs. 0.5 ± 0.2 g kg^{-1}_{DM}, respectively; Table 1; Figure 2b), and in full-sun stumps than in shade-grown stumps (0.7 ± 0.2 vs. 0.6 ± 0.2 g kg^{-1}_{DM}, respectively). No maturity × exposure interaction was observed for stump glucose concentrations.

Starch concentrations averaged 20.1 ± 21.1 g kg^{-1}_{DM}, and also had a very high CV (105%). Starch behaved very differently from the other sugars, as the treatment effect (maturity only here) was only visible in the stumps. Average starch concentrations in leaves and stems (12.0 ± 5.1 and 16.8 ± 16.8 g kg^{-1}_{DM}, respectively) were not significantly different but respectively divided by a factor close to 3 and 2 compared to the average starch concentration in stumps (32.0 ± 29.0 g kg^{-1}_{DM}; Table 1; Figure 2c,d). Very strikingly, starch concentrations

in stumps were three times higher with 5-year-old resprouts than with 2-year-old ones (46.8 ± 32.5 vs. 18.2 ± 16.1 g kg^{-1}_{DM}, respectively; Table 1; Figure 2d).

Prediction of the biochemical variables using VNIRS

As shown in Figure 3a, mean spectra differed among the three organs (leaves, stems and stumps), especially in the visible range (400-800 nm).

<Insert Figure 3 here>

A PCA was carried out on the whole spectrum set, confirming the separation of the samples according to the organ (Figure 3b), with leaves clearly separated from stems and stumps on Principal Component 1. By contrast, the spectra of the whole set were not split according to maturity or exposure (data not shown). When PCAs were built for each organ separately, the stem spectra were separated according to maturity (Figure 3c); indeed, a maturity effect was present for several stem variables (N, Glu, Fru and Suc; Table 1). By contrast, the PCA of stem spectra could not separate samples according to exposure, thereby confirming Table 1 indication that the overall effect of exposure was small. Finally, spectra of leaves and stumps could hardly be separated according to maturity or exposure (data not shown).

Considering RPD_{val} over the whole population (all organs pooled), external validation yielded (according to the criteria of Williams and Norris, 2001) excellent results for N (RPD_{val} = 11.9); good results for C:N (RPD_{val} = 5.8); fair results for starch (RPD_{val}, = 3.1); and poor results for the other variables ($RPD_{val} \leq 3$), especially for sucrose (RPD_{val} = 1.8; Table 2 and Figure 4). However, it is worth noting that for C, the error of prediction (SEPc) represented only 1% of the mean, and that poor prediction according to RPD_{val} was the effect of the low variability of C concentrations (the coefficient of variation, i.e. ratio of SD to mean, CV, was 2%).

<Insert Table 2 here>

<Insert Figure 4 here>

VNIRS predictions for the organs separately were less accurate in general because of the small size of the calibration subset (cross-validations only were carried out; Table 3). According to the RPD_{cv} criterion, the VNIRS predictions often remained fair for N, C:N, and starch ($3.1 \leq RPD_{val} \leq 4.4$ except for C:N in stumps, and for starch in leaves). Again, prediction errors for C were low (SECV < 1% of the mean), and "poor" predictions of C, according to RPD_{cv}, were mainly the effect of low C concentration variabilities (CV was 1-2%). Predictions remained poor for glucose, fructose, and sucrose. All variables except starch were less accurately predicted for stumps than for leaves and stems.

<Insert Table 3 here>

The prediction error (SEPc; or SECV for organs separately) was noticeably larger (generally four or five times) than the standard error of laboratory (SEL). SEL was 5%, 6%, and 9% for hexoses, sucrose, and starch, respectively. As the difference between prediction error and SEL is quadratic [i.e. $(\text{SEPc corrected for SEL})^2 = \text{SEPc}^2 - \text{SEL}^2$], the uncertainty of conventional determinations contributed only marginally to prediction error: correction for SEL represented 1% to 5% of SEPc or SECV in general, except for starch in leaves, stems, and stumps, and for sucrose in stems (6%, 7%, 11%, and 8%, respectively).

Discussion

NSC in coffee vegetative organs

Soluble sugars (glucose, fructose, and sucrose) and N increased upwards, while C, C:N, and starch increased downwards. The higher concentrations of N, glucose, fructose, and sucrose in leaves are consistent with the leaves' role as the main source of NSC, and likely reflect the

leaves' high metabolic rates, high concentrations of intermediary metabolites, and high proportion of living cells requiring turgor maintenance (Sala et al. 2012, Chaves et al. 2012, Sala and Mencuccini 2014). Moreover, the high concentration of N in coffee leaves (above 3%) was expected, due to the presence of caffeine.

Fructose and glucose are direct products of photosynthesis and are commonly found in significant amounts in leaves (Martínez-Vilalta et al. 2016). In accordance with the results of DaMatta et al. (2008) and Avila et al. (2020) in coffee leaves, we found that sucrose concentration was rather stable in most sampled organs. Such stability has often been related to the transport role of sucrose, because its use in sink organs is compensated by its uploading from source organs, whereas starch content is more variable (Chantuma et al. 2009, Dietze et al. 2014). In coffee in particular, leaves are able to accumulate huge amount of starch when the demand is low as compared to supply (Avila et al. 2020).

Starch accumulation in coffee stumps confirmed (i) its variability through plant ageing shown by other pioneering studies on wood of the coffee sprout (Nutman 1933, Wormer and Ebagole 1965, Patel 1970), (ii) the usual pattern of starch accumulation in trunks during vegetative phases, consistent with a reserve function in trees (Cannell and Dewar 1994, Barbaroux and Bréda 2002, Hoch et al. 2003, Silpi et al. 2007). We obtained an average starch concentration of 12 g kg^{-1}_{DM} in leaves, 17 g kg^{-1}_{DM} in stems and 32 g kg^{-1}_{DM} in stumps, which were in the same range as reported in wood trunk of other tropical trees (Latt et al. 2001, Newell et al. 2002, Silpi et al. 2007). The concentration of soluble sugars was higher in leaves than in stumps, while the contrary was observed for starch. In total, as the stump biomass was about twice that of the stems and leaves together (Defrenet et al. 2016), the estimated starch amount per plant was about twice that of soluble sugars.

Does high fruit production induce a shortage of carbohydrate and N resources in coffee trees?

Our hypothesis was that resprout exhaustion after a few productive years could be driven by a too high demand for NSC and possibly N to sustain the high fruit production during year 3 to 5. Only three variables supported this hypothesis: N, glucose, and fructose concentrations were higher in organs sampled from 2-year-old resprouts than from 5-year-old resprouts. This, together with the decrease in wood RGR in year 5, could indicate a less active photosynthesis in older shoots (Charbonnier et al. 2017). Lower N content in leaves in year 5 could, in particular, be linked to a high transfer of N to fruits (Avila et al. 2020). However, three other variables invalidated that hypothesis; namely C, C:N, and starch, the latter being by far the most quantitatively represented NSC in coffee trees. In other words, when bearing young and unproductive 2-year-old resprouts, the coffee plants had more available N in the leaves and maintained higher soluble sugars concentration, while in the old and mature stage (5-year-old resprouts), coffee plants had less N in leaves, and accumulated starch in the lower organs.

Could this difference in concentrations originate from dilution effects only? Indeed, a smaller canopy at 2-year-old, with similar N availability from the soil could express higher concentrations of N and soluble sugars. However, since a higher starch concentration in stumps at 5-year-old cannot be the consequence of dilution effects, there was actual starch accumulation from n+2 to n+5. It was likely originating from the flux of soluble sugars (glucose, sucrose and less clearly fructose) that were more concentrated in stem at 2 years old, and then accumulated as starch in stump at 5 years old.

Hence, high fruit production did not induce total NSC drop, but occurred simultaneously with the transfer of soluble sugars from stems to stumps, and with starch build-up in the stumps. The decrease in resprout RGR could not be linked to a shortage of NSC resource at the plant

scale and the same conclusion stands for the subsequent collapse in fruit load. Actually, the existence of a large, hidden sink forming during oldest stages of coffee resprouts (from 4 to 5 years-old) was suspected by our previous study (Charbonnier et al. 2017). This sink was hypothesized from a decrease of Net Assimilation Rate (NAR) at that stage. Charbonnier et al. (2017) proposed that photosynthetic products could be diverted from the expected new shoot flush, and translocated elsewhere. Here, we confirm this hypothesis: starch did accumulate in perennial organs like stumps, and probably belowground as well (indeed, Martínez-Vilalta et al., 2016 showed that starch accumulation could even be greater in roots than in stump). Given such pattern, NSC content at plant scale or in the woody perennial parts may not reflect short-term C balance of the shoots.

The increase in starch content in stump between year 2 and year 5 can be linked to the response to pruning. The initial growth of the resprouts created a high NSC demand that could be sustained only by the use of reserves located in perennial parts (stump and roots), as there was no leaf to provide photo-assimilates in year 1. This consumed a part of the accumulated starch that can explain the low starch content in year 2. Once the new resprouts were grown and photosynthetic activity was restored, the coffee trees refilled the depleted starch reserves in the stump wood, a process that occurred at the same time as fruit production and vegetative growth, leading to higher stump starch content in year 5. It is now widely accepted that NSC reserves in trees serve mainly in a survival perspective, to face possible prolonged shortage of assimilates that can occur following adverse climatic conditions or pest-induced defoliation (Hoch et al. 2003, Sala et al. 2012). Therefore, trees need to refill their reserves whenever they get low even if NSC demand may be high at the same time for other sinks such as vegetative growth or reproduction (Barbaroux and Bréda 2002, Silpi et al. 2007, Sala et al. 2012).

Moreover, pruning creates a wound stress that is known to increase allocation towards reserves on the long term, as shown following artificial defoliation (Wiley et al. 2013), or tapping of rubber trees for latex production (Silpi et al. 2007, Chantuma et al. 2009). It was shown that repeated pruning may stimulate the storage of reserve in tree wood (Clair-Maczulajtys et al. 1999).

Our results could be compared to several recent results showing that reserves are seldom mobilized for fruit production, which would rely mainly upon recent assimilates (Bustan et al. 2011, Hoch et al. 2013, Ichie et al. 2013). This behaviour suggests a decoupling between the NSC needs of the old resprout and those of the perennial stump. Indeed, Charbonnier et al. (2017) pointed out that allocation to coffee wood was little influenced by biennial bearing: although leaf development was low in years with high fruit demand, allocation to wood decreased only slightly. Vezy et al. (2020) used this observation as a modelling assumption to allocate C to wood in priority in their model and successfully simulate growth of every single coffee organ. Indeed, to achieve the multi-objective calibration of the model, reserves had to fluctuate seasonally, with a minimum simulated during grain filling, consistently with our assumption that reserves should reach a minimum in October. Similar findings were shown for other alternate fruit bearing species (Bustan et al. 2011).

Does shade change NSC and nitrogen partitioning?

Globally, the exposure effect was much smaller than the organ and maturity effects. That result invalidated the hypothesis that full sun and shade induce different allocation of N and NSC, at least in the conditions of our study where the nutrient inputs to the crop were optimal. Indeed, exposure affected only three variables (C, glucose, and fructose), which were higher in full sun, indicating that source effect actually prevailed over the sink effect. Exposure had no significant effect upon starch, suggesting that allocation to starch was not an adjustment

variable. In studies upon the same plot, Charbonnier et al. (2017) reported that shade (here 70% of light reduction, just below the crown) had no effect upon specific leaf area, NPP, and NAR. Those authors stressed that a sharp reduction of absorbed light (aPAR) and photosynthesis under shade was compensated by increased light-use efficiency and that overall, NPP was not affected under shade.

Some applications for dynamic coffee-crop modelling

Dynamic modelling of perennial plants often assumes a reserve pool receiving only the leftovers from daily allocations, and re-injects a fraction into gross primary production (GPP) at the next time step. However, more recent studies indicate that such view is unlikely. Authors of those studies considered, as an alternative, that reserves are a sink competing with others, and not necessarily with lower priority than vegetative growth (Silpi et al. 2007, Chantuma et al. 2009, Genet et al. 2010, Sala et al. 2012, Vezy et al. 2020). Actually, if this was not the case, trees would not be able to cope with unpredicted events such as a destruction of their foliage by a herbivore (Dunn et al. 1990, Guérard et al. 2007). Our results are consistent with such concept, as starch accumulated in the stump late in the resprout lives, even as resource supply seemed to become limiting at the leaf level (5-year-old resprouts had lower N concentrations in leaves, and lower soluble sugar concentrations in stems). An alternative hypothesis is that, as high fruit demand induces a strong transfer of N from leaves to fruits (Avila et al. 2020), reduced N availability for growth could lead to an excess of C, which could not be used for growth and would therefore accumulate in long-term storage organs. However, according to Avila et al. (2020) this should also lead to increased starch content in leaf, which was not observed in the present study.

Most dynamic models also link C and N cycles through C:N, and assume that C:N is a constant per organ. However, in our results C:N was significantly lower in 2-year-old than in 5-year-old leaves and stems, indicating that such model assumption should be revised as well.

Evolutionary considerations: from lodging in the wild to exhaustion in the field?

We suggest that competition among the stump, leaves, and fruits could be the remnant of an adaptation of *Coffea arabica* to the understorey environment of Eastern African forests, where the coffee plant has evolved in reduced-light conditions, and is prone to lodging (strong bending leading to the death of the shoot) and resprouting. Resprouting ability was shown to be higher in shade-tolerant woody species of the tropics, traducing an adaptation to stresses induced by damage from falling debris (Poorter et al. 2010). According to the "life history theory" (Poorter et al. 2010) species adapted to low-resources conditions (e.g. growing under shade) should have a high inherent survival rate but low growth rate. Resprouting is a typical adaptation to low-resource conditions or high occurrence of disturbances that increases such survival rate to the detriment of growth (Clarke et al. 2013).

Resprouting after stress or after lodging is mediated by accumulation of reserves in well-protected organs below-ground, but also by formation of a high-density wood (to prevent diseases), and production of numerous axillary dormant buds (Poorter et al. 2010). All three of these mediation mechanisms are reasonably common in coffee. Natural lodging would precede the regeneration of coffee trees in the wild. In Ethiopian forests devoid of management, coffee plants can often reach a height of four or five meters, or even more (Sylvain 1955). After lodging, stems resprout from the stump base, allowing the coffee tree to recover. We argue here that such resprouting following lodging likely requires previous accumulation of reserves in the stump, such as after pruning coffee trees under cultivation. Such a peculiar behaviour could have been transmitted to modern cultivars.

Possible consequences for coffee plant management and breeding.

As the collapse in fruit production after 3 years of high yield does not seem related to a limitation in C resource, it is not likely that measuring NSC in the short-lived organs (leaves, shoots) would provide indicators of resprout yield potential nor indicate the right time to prune them selectively. Given that the number of fruits depends directly upon the number of vegetative nodes initiated during the previous year (Cerda et al. 2017), the fine observation of shoot development in the late years remains an effective way to predict fruit production collapse. Shoot growth and decay may not be directly correlated to NSC availability, even locally (Chaves et al. 2012). However, starch was confirmed here to be a crucial variable, particularly in the perennial parts of the plant. The dynamics of starch should be monitored more precisely during a complete cycle, particularly to determine when it starts accumulating and if it is prone to seasonal or inter-annual fluctuations. The root systems should be investigated as well, since it could contribute largely to the total reserve amount. Experiments with variable pruning frequency and over several pruning cycles would also confirm whether reserve accumulation increases in response to this repeated perturbation. Actually, if high starch levels are positive to support resprouting and may be considered as a safety tank for sustainable yield on the long run, one can wonder if, in relation to its natural behaviour in forest understorey, the coffee plant does not accumulate more NSC reserves than necessary in a well-controlled farm environment. The trade-off between persistence (ability to resprout, Clarke et al. 2013) and reproduction, inherited from the understorey origin of the species, may restrict coffee bean production. It is suggested here that selecting varieties with lower allocation of NSC to the starch sink would lengthen the lifespan of resprouts, and improve the ratio of productive to unproductive years. In addition, the use of this selection criterion may

reduce labour costs associated with pruning, and with resprout selection after resprouting. But this could also come to the detriment of long-term survival.

The fact that shading did not affect much NSC resource and total N confirmed the interest of associating shading trees to coffees in suboptimal climatic conditions, as NPP and yield were not much affected (Charbonnier et al. 2017). However, it is also not likely that shading would improve the productive span of coffee resprouts in such conditions.

VNIRS accuracy for predicting biochemical variables

Overall, good VNIRS prediction was always achieved for N, whatever the set (whole set or organs separately), especially when the variability was high (whole population), but also when it was low (for leaves, CV was 9%). This finding suggests that in the samples considered here, N compounds had a clear and specific VNIRS signature, which is consistent with the fact that bands at 1500-1530, 2050-2060 and 2168-2180 nm have been assigned to proteins (Workman and Weyer 2008). Fair VNIRS prediction was achieved for C:N in general, except for stumps, possibly due to error propagation (for C:N, the reference was calculated, not measured). In contrast, C concentration, which had limited variations (from 414 to 486 g kg^{-1}_{DM}), was poorly predicted according to RPD_{val}, although the prediction error was small (standard error < 5 g kg^{-1}_{DM} and ≤ 2% of the mean).

Quantification of N and C concentrations in plant vegetative parts using VNIRS has been reported by many authors for various plants: tomato, cotton, pine needles, rice, and wheat (Batten et al. 1991, Gillon et al. 1999, Riley and Cánaves 2002, Morón et al. 2007, Ulissi et al. 2011). To our knowledge, such studies have not been made on coffee plant vegetative organs. However, it is worth noting that the performances of the VNIRS predictions of C and N reported for different plants in the cited papers were similar to those achieved here on aerial vegetative parts of coffee, for the same concentration ranges.

In the present study, VNIRS prediction of starch content was fair for all sets except in leaves, which had lower starch concentrations than the other organs (12 vs. 17-32 g kg^{-1}_{DM} on average, respectively). Prediction accuracy (SEPc = 7 g kg^{-1}_{DM}) was comparable to or sometimes better than that reported for starch concentration in other plants (Card et al. 1988, Curran et al. 1992, Lomborg et al. 2010, Ramirez et al. 2015): as for N content, this result suggests that starch has a clear and specific VNIRS signature. For instance, bands at 1450 and 2280 nm have been assigned to starch (Workman and Weyer 2008). VNIRS could thus become a preferred tool to screen for N and starch contents of different coffee varieties, or during pruning cycles. Prediction was less accurate for glucose, fructose, and sucrose, which had low concentrations in general (< 13 g kg^{-1}_{DM} on average, except for sucrose in leaves, which reached 20 g kg^{-1}_{DM} on average).

The uncertainty of VNIRS predictions could be attributed only marginally to the uncertainty of reference sugar analyses. It is more likely attributable to the low specificity of the VNIRS signature of the sugars considered, which do not differ much in their composition. Very few papers have reported near-infrared spectroscopy (NIRS) quantification of individual sugars in plant vegetative parts. Ramirez et al. (2015) reported that RPD_{val} = 3.3 for the sum of glucose, fructose, and sucrose concentrations in a diverse set of samples originating from four organs of numerous tree species. That result is more accurate than ours, which were obtained for individual sugars in the single species *Coffea arabica* L. var. Caturra (RPD_{val} = 1.8-2.8 over the total set). Piaskowski et al. (2016) achieved RPD_{cv} = 2.2 for soluble carbohydrates in immature wheat stems (vs. 1.8-2.8 and 1.5-1.9 here for individual sugars in the total and stem sets, respectively), but for a much wider range of concentrations than in the present study: mean (± SD) = 283 (± 76) vs. 22 (± 14) g kg^{-1}_{DM}, respectively.

To conclude, according to the prediction results of the present study, VNIRS seems to be more interesting when applied at the plant scale than at the organ scale. It particularly

represents an accurate alternative for the N and starch content assessment in the different organs of coffee trees. Before routine use at the plot scale, it would first be necessary to fill the existing database and recalibrate the models with new samples (characterized by spectra and conventional analyses) collected at different months and over several years, which would allow to include the time variable in the prediction model. However, concerning the other NSC, as predictions were not accurate, it may be more appropriate in further studies, to predict the sum of glucose, fructose and sucrose contents and evaluate this model accuracy. Indeed, as mentioned before, a common spectral signature may exist between these soluble sugars but without specific distinction, as they are very similar in their composition. Finally, concerning C content, the too low variability of this variable in the dataset induced a limit in model accuracy; thus, collecting new data at different times (as for N and starch contents) might allow the dataset to include higher variations and help the model to provide lower SEP_c compared to SD.

Summary and conclusions

This study, conducted in the field, on mature plants of *Coffea arabica* var. Caturra grown in optimal nutrient conditions, addressed the effects of high fruit production on non-structural carbohydrate (NSC) and nitrogen (N) resources.

We invalidated the hypothesis of resource depletion on a plant scale through successive years of high yield. We also invalidated the hypothesis that there could be substantial differences in resource balance between plants grown in full sun versus in shade. Indeed, we found out that starch accumulated in the perennial stump when fruit yield was maximum. Hence, there is no resource imbalance, rather a triple competition (among fruits, new shoot growth, and starch accumulation). Such behavior could be interpreted as a relic from the lodging behavior

observed in wild coffees, sustaining the ability to resprout. Measuring starch in stumps could be useful to evaluate the resprouting potential of coffee trees, and therefore their long-run yield potential. It may also be used for breeding strategies aiming at increasing the allocation of resources towards the productive resprouts.

VNIRS proved to be suitable for this task. It was also efficient for quantifying N concentration, and to a lesser extent C concentration and C:N, but not for quantifying glucose, fructose, and sucrose.

Conflict of interest

None declared

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Figure legends

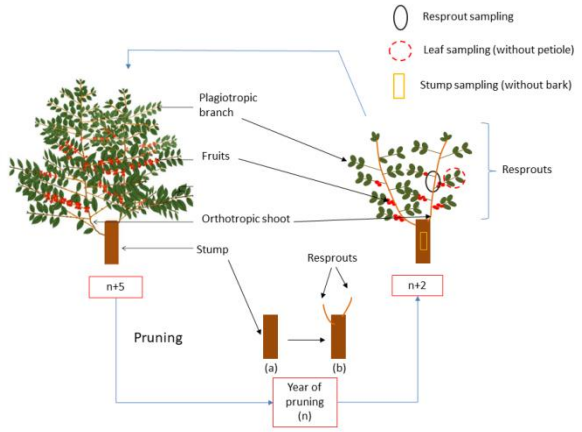

Figure 1. Description of (i) the structure of *Coffea arabica L.* for three resprout ages (0, 2 and 5 years old), (ii) the management methods of coffee tree in the studied area and (iii) the sampling procedure for the three studied organs (leaves, stem, stump). Blue dashed arrows illustrate one pruning/resprouting cycle. Drawing of the 5 years old tree courtesy to Simon Vonthron.

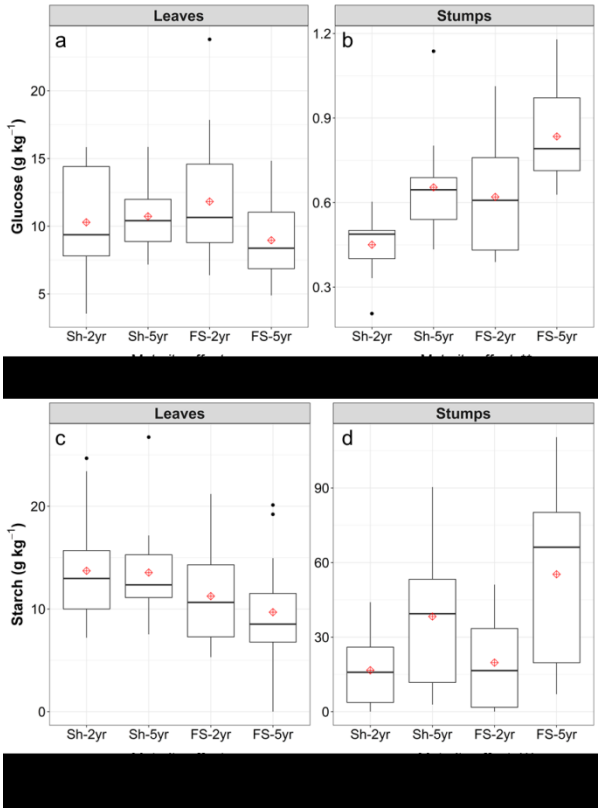

Figure 2. Distributions of glucose (as an example for soluble non-structural carbohydrates-NSC; Figures a and b) and starch (as an example of reserve NSC; Figures c and d) in two contrasted coffee organs: leaves and stumps (stems behaved in an intermediate way). For each case, data are given according to exposure (shade-Sh vs. full sun-FS) and maturity (2-year-old vs. 5-year-old) and significant effects of both factors were obtained using *glm* models (cf. Table 3). Significance levels: ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; n.s.: not significant. Vertical lines inside boxes represent medians, red diamonds means. The bottom and top of each box are first and third quartiles. Boxplot whisker ends are either the minimum and maximum values when they were included in 1.5 times the interquartile range; otherwise, the latter value was used (and the minimum and/or maximum points were represented out of the boxplot). Coffee agroforestry plot, Costa Rica.

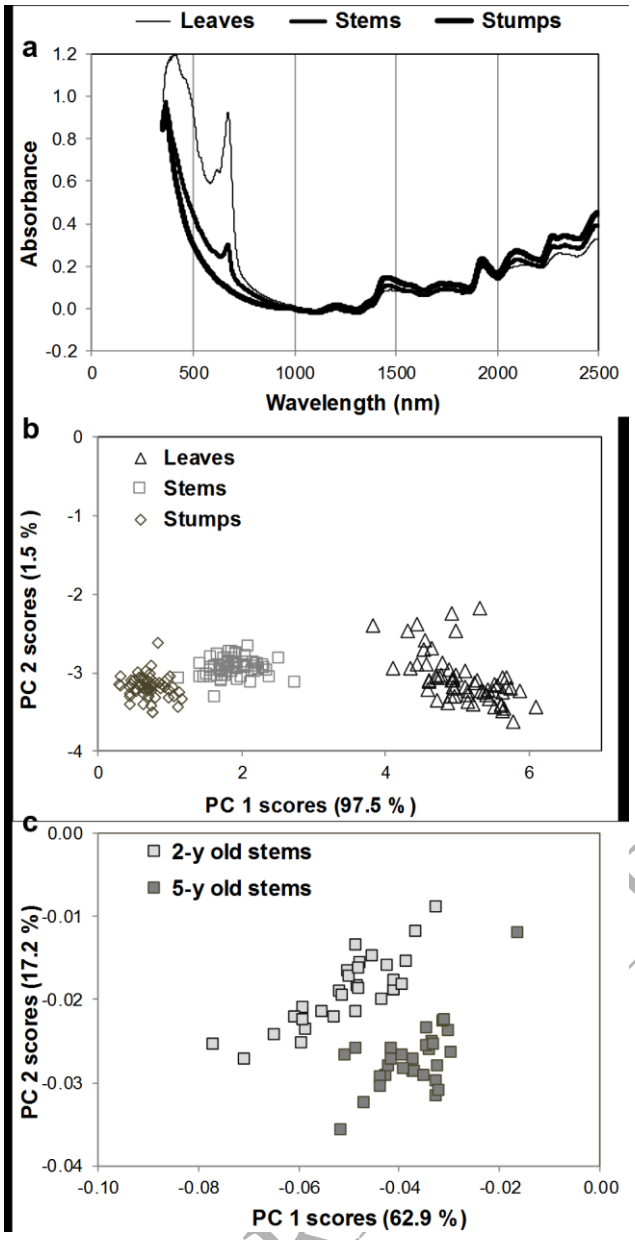

Figure 3. VNIR data per organ: a/ mean VNIR spectrum of coffee leaves, stems and stumps; b/ projection of the sample spectra on the first two principal components (PC) of the Principal Component Analysis (PCA) performed on the whole set of raw absorbance spectra (None001) and according to organ (N = 180 samples); c/ projection of the stem spectra on the first two PC of the PCA performed after first derivation (None144), and according to maturity (N = 60). Coffee agroforestry plot, Costa Rica.

Figure 4. Comparison between reference determinations and VNIRS predictions for the seven studied variables over the external validation set (all organs pooled), using the most appropriate pre-processing method (SNV001 for C:N and fructose, SNVD001 for C and N, SNV144 for glucose and starch, and SNVD244 for sucrose). N = 80 samples. Coffee agroforestry plot, Costa Rica.

Table 1. Conventional determinations of C, N, C:N ratio, Glu, Fru, Suc and Sta. Results are from the whole set of 178 samples, and are presented in the format "Mean (Standard Deviation; SD)". The table shows the results of the mean comparisons between i) the three organs (*glm* followed by Tukey-Kramer test), as well as ii) the maturity treatments, iii) the exposure treatments, iv) the Exposure × Maturity interaction (written as M × E), studied in each organ (*glm*). Significance levels: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; n.s. not significant. When any effect is significant, the stars (*) are attributed to the treatment presenting the highest value for the variable of interest.

Varia-ble	Organ	All treatments		Maturity		Exposure		Organ effect	Maturity effect		Exposure effect		M x E
		Mean (SD) g kg^{-1}_{DM}	CV (%)	2yr-old g kg^{-1}_{DM}	5yr-old g kg^{-1}_{DM}	Shade g kg^{-1}_{DM}	Full Sun g kg^{-1}_{DM}		2yr-old	5yr-old	Shade	Full Sun	-
C	All	467 (10.1)	2	463 (9.7)	470 (9.6)	464 (10.5)	469 (9.1)	-					
	Leaves	463 (10.4)		457 (8.7)	468 (9.3)	458 (8.2)	467 (10.5)	B		**		*	
	Stems	463 (9.9)		459 (4.3)	468 (12.0)	461 (11.3)	465 (8.0)	B					
	Stumps	474 (4.4)		474 (4.4)	474 (4.4)	473 (3.7)	475 (4.9)	A***				*	
N	All	17.0 (10.5)	62	18.4 (11.0)	15.6 (9.9)	17.0 (10.5)	16.9 (10.7)	-					
	Leaves	31.0 (2.6)		33.0 (1.8)	29.1 (1.7)	30.9 (2.3)	31.2 (2.9)	A***	***				
	Stems	12.5 (2.9)		15.1 (1.4)	9.9 (1.2)	12.6 (3.3)	12.4 (2.6)	B	***				*
	Stumps	7.1 (1.1)		7.0 (1.2)	7.2 (0.9)	7.2 (1.1)	6.9 (1.0)	C					
C:N	All	40.6 (23.3)	57	38.0 (24.4)	43.2 (21.9)	40.0 (22.7)	41.2 (23.9)	-					
	Leaves	15.0 (1.5)		13.9 (0.8)	16.2 (1.1)	14.9 (1.3)	15.1 (1.7)	C		***			
	Stems	39.3 (9.8)		30.7 (3.0)	47.8 (6.2)	39.0 (10.1)	39.6 (9.8)	B		***			
	Stumps	68.4 (10.2)		69.4 (11.5)	67.2 (8.5)	66.9 (9.7)	69.9 (10.6)	A***					

Glu	All	4.4 (4.9)	110	4.7 (5.3)	4.2 (4.5)	4.3 (4.9)	4.6 (4.9)	-			
	Leaves	10.4 (3.7)		11.1 (4.4)	9.8 (2.9)	10.5 (3.4)	10.4 (4.1)	A***			
	Stems	2.1 (1.0)		2.5 (1.2)	1.8 (0.8)	1.8 (1.1)	2.4 (0.9)	B	**		
	Stumps	0.64 (0.21)		0.54 (0.18)	0.74 (0.19)	0.55 (0.17)	0.72 (0.21)	C		**	**
Fru	All	4.4 (4.7)	108	4.5 (4.9)	4.2 (4.6)	4.3 (4.8)	4.5 (4.6)	-			
	Leaves	10.1 (3.7)		10.2 (4.1)	9.9 (3.3)	10.3 (3.4)	9.8 (4.0)	A***			*
	Stems	2.4 (1.5)		2.9 (1.8)	1.9 (0.9)	2.0 (1.5)	2.8 (1.3)	B	*		
	Stumps	0.53 (0.22)		0.46 (0.18)	0.61 (0.23)	0.44 (0.13)	0.63 (0.25)	C			**
Suc	All	13.3 (7.4)	55	12.7 (7.7)	14.0 (7.0)	12.4 (6.3)	14.3 (8.2)	-			
	Leaves	19.5 (8.9)		18.2 (10.2)	20.8 (7.4)	18.0 (7.2)	21.0 (10.2)	A***			
	Stems	11.4 (3.1)		12.8 (2.5)	10.1 (3.1)	11.1 (3.0)	11.7 (3.2)	B	**		
	Stumps	8.9 (3.4)		7.0 (2.7)	10.9 (3.1)	7.9 (2.4)	9.8 (4.0)	C		**	
Sta	All	20.2 (21.1)	105	15.1 (12.2)	25.3 (26.4)	18.6 (17.5)	21.7 (24.1)	-			
	Leaves	12.0 (5.1)		12.5 (5.0)	11.6 (5.2)	13.6 (4.8)	10.5 (5.0)				
	Stems	16.8 (16.8)		14.7 (12.5)	19.0 (20.2)	15.5 (15.2)	18.2 (18.3)	n.s.			
	Stumps	32.0 (29.0)		18.2 (16.1)	46.8 (32.5)	27.1 (24.3)	36.9 (32.7)			***	
Total NSC	All	42.3 (22.7)	54	37.0 (18.8)	47.7 (25.1)	39.6 (20.9)	45.0 (24.2)	-			
	Leaves	52.0 (10.4)		51.9 (12.1)	52.2 (8.7)	52.3 (9.8)	51.8 (11.2)	A***			
	Stems	32.8 (19.0)		32.8 (16.1)	32.7 (21.8)	30.4 (18.0)	35.1 (20.1)	B			
	Stumps	42.1 (30.3)		26.2 (17.7)	59.0 (32.1)	36.0 (25.7)	48.1 (33.7)	B		**	

Table 2. VNIRS results from calibration (with cross-validation) over the three organ sets, using the pre-processing methods that yielded the best cross-validation results (all variables in g kg^{-1}_{DM}, except C:N). None corresponds to no spectral transformation, SNV to standard normal variate transformation and SNVD to both SNV and de-trending; these spectral transformations are either associated to no derivatives and no smoothing (001), or first derivatives with 4-point

gap and 4-point smoothing (144) or second derivatives with 4-point gap and 4-point smoothing (244).

Organ	Variable	Pre-processing	N_{os} [a]	Calibration (with cross-validation)						
				N_{oc} [b]	N_{cal} [c]	Mean	SD [d]	SECV [e]	R^2_{cv} [f]	RPD_{cv} [g]
Leaves	C	None001	0	3	58	463	10	4	0.85	2.6
	N	SNVD001	0	2	59	31.0	2.7	0.8	0.90	3.2
	C:N	SNVD001	0	1	60	15.0	1.5	0.5	0.90	3.2
	Glu	None001	0	3	58	10.2	3.2	2.1	0.58	1.5
	Fru	SNVD001	0	2	59	10.0	3.3	2.3	0.51	1.4
	Suc	SNVD144	0	2	59	19.1	8.8	4.2	0.78	2.1
	Sta	SNVD001	0	4	57	11.9	4.6	3.1	0.56	1.5
Stems	C	None244	0	4	56	465	7	3	0.84	2.5
	N	None001	1	2	57	12.6	2.8	0.7	0.95	4.3
	C:N	SNV001	1	4	55	37.9	8.3	2.1	0.94	4.0
	Glu	SNVD144	0	3	57	2.1	1.0	0.5	0.73	1.9
	Fru	SNVD001	1	0	59	2.4	1.5	1.0	0.56	1.5
	Suc	SNVD144	0	2	58	11.4	3.1	1.8	0.66	1.7
	Sta	SNV001	1	2	57	16.4	16.0	4.2	0.93	3.8
Stumps	C	None144	0	6	53	474	4	2	0.64	1.7
	N	None001	2	3	54	7.1	1.1	0.3	0.89	3.1
	C:N	SNV001	1	5	53	67.2	8.7	3.8	0.81	2.3
	Glu	None001	2	3	54	0.6	0.2	0.2	0.52	1.4
	Fru	None244	0	1	58	0.5	0.2	0.2	0.17	1.1
	Suc	None001	2	4	53	8.7	3.2	2.3	0.48	1.4
	Sta	SNV144	0	6	53	32.2	27.3	6.2	0.95	4.4

[a] Number of spectral outliers.
[b] Number of calibration outliers.
[c] Size of the calibration subset.
[d] Standard deviation of the subset.
[e] Standard error of cross-validation (in the unit of the variable).
[f] Determination coefficient for cross-validation.
[g] Ratio of calibration subset SD to SECV (unitless)

Table 3. VNIRS results from calibration (with cross-validation) over the three organ sets, using the pre-processing methods that yielded the best cross-validation results (all variables in g kg^{-1}_{DM}, except C:N). None corresponds to no spectral transformation, SNV to standard normal variate transformation and SNVD to both SNV and de-trending; these spectral transformations

are either associated to no derivatives and no smoothing (001), or first derivatives with 4-point gap and 4-point smoothing (144) or second derivatives with 4-point gap and 4-point smoothing (244).

Organ	Variable	Pre-processing		Calibration (with cross-validation)						
			N_{os} [a]	N_{oc} [b]	N_{cal} [c]	Mean	SD [d]	SECV [e]	R^2_{cv} [f]	RPD_{cv} [g]
Leaves	C	None001	0	3	58	463	10	4	0.85	2.6
	N	SNVD001	0	2	59	31.0	2.7	0.8	0.90	3.2
	C:N	SNVD001	0	1	60	15.0	1.5	0.5	0.90	3.2
	Glu	None001	0	3	58	10.2	3.2	2.1	0.58	1.5
	Fru	SNVD001	0	2	59	10.0	3.3	2.3	0.51	1.4
	Suc	SNVD144	0	2	59	19.1	8.8	4.2	0.78	2.1
	Sta	SNVD001	0	4	57	11.9	4.6	3.1	0.56	1.5
Stems	C	None244	0	4	56	465	7	3	0.84	2.5
	N	None001	1	2	57	12.6	2.8	0.7	0.95	4.3
	C:N	SNV001	1	4	55	37.9	8.3	2.1	0.94	4.0
	Glu	SNVD144	0	3	57	2.1	1.0	0.5	0.73	1.9
	Fru	SNVD001	1	0	59	2.4	1.5	1.0	0.56	1.5
	Suc	SNVD144	0	2	58	11.4	3.1	1.8	0.66	1.7
	Sta	SNV001	1	2	57	16.4	16.0	4.2	0.93	3.8
Stumps	C	None144	0	6	53	474	4	2	0.64	1.7
	N	None001	2	3	54	7.1	1.1	0.3	0.89	3.1
	C:N	SNV001	1	5	53	67.2	8.7	3.8	0.81	2.3
	Glu	None001	2	3	54	0.6	0.2	0.2	0.52	1.4
	Fru	None244	0	1	58	0.5	0.2	0.2	0.17	1.1
	Suc	None001	2	4	53	8.7	3.2	2.3	0.48	1.4
	Sta	SNV144	0	6	53	32.2	27.3	6.2	0.95	4.4

[a] Number of spectral outliers.
[b] Number of calibration outliers.
[c] Size of the calibration subset.
[d] Standard deviation of the subset.
[e] Standard error of cross-validation (in the unit of the variable).
[f] Determination coefficient for cross-validation.
[g] Ratio of calibration subset SD to SECV (unitless)