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Spatial variability in carbon and nitrogen related traits in apple trees: the effects of the light environment and crop load

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HIGHLIGHTS

Acclimation of leaf traits to light in apple trees depends on the fruit demand for carbon and is negligible when fruit loads are small

ABSTRACT

Photosynthetic carbon assimilation rates are highly dependent on environmental factors such as light availability and on metabolic limitations such as the demand for carbon by sink organs. The relative effects of light and sink demand on photosynthesis in perennial plants such as trees remain poorly characterised. The aim of the present study was therefore to characterize the relationships between light and fruit load on a range of leaf traits including photosynthesis, nonstructural carbohydrate (*NSC*) contents, leaf structure and nitrogen-related variables in fruiting ('ON') and nonfruiting ('OFF') 'Golden Delicious' apple trees. We show that crop status (at the tree scale) exerts a greater influence over leaf traits than the local light environment, or the local fruit load. High rates of photosynthesis were observed in the ON trees. This was correlated with a high leaf nitrogen content. In contrast, little spatial variability in photosynthesis rates was observed in the OFF trees. The lack of variation in photosynthesis rates was associated with high leaf NSC contents at the tree level. Taken together, these results suggest that low carbon demand leads to feedback limitations on photosynthesis resulting in a low level of within-tree variability. These findings provide new insights into carbon and nitrogen allocations within trees that are heavily dependent on carbon demand.

INTRODUCTION

Within-plant microclimate gradients, such as those due to incoming light, air temperature, humidity, and wind, locally affect organ function and structure. Leaves respond to multiple constraints in various ways; these responses were gathered into a framework built over a large range of species, the so-called "leaf economics spectrum" (Wright *et al*., 2004; Niinements *et al*., 2015). Leaves acclimate to changing light throughout plant development. Light acclimation has been intensively studied for decades, as incoming light is one of most important resources for leaf net carbon (C) assimilation (A_{net}) and energy balance. In trees, numerous studies have reported decreasing A_{net} from the upper to lower layers of forest ecosystems. The authors have linked these photosynthetic profiles to available light and traits involved in light harvesting and photosynthetic capacities, such as nitrogen (N) content per unit area (Hollinger, 1996; Makela *et al*., 2008; Mc Murtrie and Dewar, 2011; Niinemets *et al*., 2004; Thornley, 2004). These relationships have led to "the optimization hypothesis". Following this hypothesis, the C and N economy is oriented towards investment of leaf N and leaf biomass for maximum light interception and C return on protein invested (Mooney, 1979; Bloom, 1985). This optimization has also been considered in models that account for within-tree N distribution and leaf gas exchanges that depend on incoming radiation at the individual scale (e.g., Le Roux *et al*., 2001, Prieto *et al*., 2012; Buckley *et al*., 2013) and in ecosystem models at a larger scale (Davi *et al*., 2005; Franklin, 2007). However, within that framework, both the leaf biochemical parameters driving variations in A_{net} and stomatal conductance are assumed to respond to N allocation and abiotic factors only. Therefore, the influence of endogenous factors such as source:sink relationships on the acclimation of leaves to light gradients has been much less investigated, even if they appear to impact many C- or N-related processes at the plant scale (Quilot *et al*. 2004; Urban *et al*. 2004, Poirier-Pocovi *et al*. 2018).

The lack of optimal distribution of A_{net} and its associated traits within trees has been noted in some studies, and nonoptimal distribution is explained by unidentified constraints that could involve source:sink relationships (Niinemets, 2012; Buckley *et al*., 2013; Hirosaka, 2014). There is a growing body of compelling evidence suggesting that, at the tree scale, leaf C function is strongly sink-driven. This phenomenon is usually observed through the downward regulation of A_{net} when carbon demand is low (Sheen, 1994; Paul and Foyer, 2001; Quentin *et al*., 2013; Pallas *et al*. 2018). This decrease in A_{net} is associated with a change in nonstructural carbohydrate (*NSC*, mostly starch and soluble sugars) turnover,

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which leads to a global increase in *NSC* in leaves when the C demand of reproductive organs is low or on girdled branches (Nebauer *et al*., 2011; Poirier-Pocovi *et al*., 2018; Urban *et al*. 2004; 2007). This adjustment of photosynthetic capacity to carbon demand and storage capacity can override the direct short-term control of photosynthesis by light and CO_2 (Paul and Foyer, 2001).

Fruit trees are of major interest for studying the impact of source-sink relationships on carbon metabolism because they are naturally prone to biennial bearing, which leads to contrasting intensities of competition for C between organs, which depends on the year (Monselise and Goldschmidt 1982). Moreover, in fruit trees, the local effect of fruit on the A_{net} of nearby leaves has also been noted (e.g., Bairam *et al*., 2019; Gucci *et al*., 1995; Palmer *et al*., 1991; Saa and Brown, 2014; Syvertsen *et al*., 2003), even though a tree-scale effect of fruit presence has also been shown (Palmer *et al*., 1991; Walcroft *et al*., 2004; Pallas *et al*., 2018; Belhassine *et al*. 2019). It is thus still unclear at which spatial scale the downregulation of leaf A_{net} by *NSC* contents is exerted: the tree scale (*via* the crop load of the whole tree) or at the local (shoot) scale (through local fruit).

Photosynthetic-associated variables also interact with crop load and C demand at different scales of tree organization (Gucci *et al*., 1995; Urban *et al*., 2004; Palmer *et al*., 1991; Saa and Brown, 2014). In that respect, leaf N increases with crop load (Urban *et al*., 2004; Stander *et al*., 2017), presumably because the amount of N absorbed by roots increases (Ding *et al*., 2017). In some cases, competition for N allocation between a growing shoot that bears fruit and a nearby vegetative shoot may decrease A_{net} in the nearby leaves by reducing their N content (Saa and Brown, 2014 on almond trees). The light gradient within a single tree is another cause of major variation in leaf N. The latter can be expressed through either the bulk N concentration (per dry mass basis, *Nm*) or variations in leaf mass per area (*LMA*, if leaf N is expressed per unit area, *Na*), with *LMA* being the ratio between the leaf dry weight (*LDW*) and the leaf surface area (*La*). As *LMA* is also positively correlated with light exposure (Poorter *et al*., 2019), it is thus considered the main driver for increasing A_{net} among and within species (Niinemets, 1999). The influence of *LMA* is considered greater than leaf N content and is related to seasonal changes in functional and structural A_{net}-related traits (Montpied *et al*., 2009). However, little is known about *LMA* variation as a function of crop load. *LMA* is reduced for fruiting orange trees compared with 50% defruited trees and for leaves of fruiting shoots compared with those of nonfruiting ones (Syvertsen *et al*., 2003).

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Conversely, Urban *et al*. (2004) did not observe a variation in *LMA* with crop load for mango trees.

This study aims to determine how the spatial variability resulting from local acclimation to intercepted light could be modulated by source-sink relationships. More precisely, we studied 'Golden Delicious' apple trees with either high or low crop loads. For these trees, we concomitantly assessed the impacts of intercepted light and crop load on within-tree spatial variability in (i) leaf photosynthesis, (ii) leaf structural traits (*LMA*, leaf weight), (iii) N-related traits (*Na*, *Nm* and chlorophyll concentration), and (iv) *NSC* contents. Furthermore, we analyzed fruiting and nonfruiting shoots to clarify the scale (shoot or tree) at which the source:sink relationships regulate leaf trait acclimation to a light gradient.

MATERIALS AND METHODS

Study site and meteorological conditions

This study was conducted at the SudExpé experimental orchard in Marsillargues, France (43"66′N 4"18′E). The orchard was planted in 1998 with the 'Tentation' cultivar grafted on 'Pajam2' rootstock, which was then top grafted with 'Golden Delicious' in 2005. The experiment was performed on Solaxe trained trees (Lauri and Lespinasse, 1998). The trees were pruned and thinned according to commercial practices until the beginning of the experiment. In both years of the study (2016 and 2017), all the flowers were removed two weeks after full bloom on some of the trees ('OFF' trees), while the others were not subjected to any thinning ('ON' trees).

Light microclimate monitoring, photosynthesis measurements and sampling of plant material were performed during early July 2016, late July 2016, mid-August 2016 and late July 2017 (Table 1). Climatic variables were recorded in the vicinity of the trees by a weather station located at a height of 2.5 m. Climatic conditions were similar in the four periods, with a warm and dry climate. The mean daily temperature and relative humidity were 24.3 °C and 57.7%, respectively, with mean daily maximum and minimum temperatures of 31.5 °C and 17.5 °C and mean daily maximum and minimum relative humidities of 86.1% and 32.9%, respectively (Supplementary material Table S1). The daily photosynthetically active radiation (*PAR*) intensity slightly differed between periods and ranged from 43.3 to 53.7 mol m^{-2} day^{-1}, likely due to variations in photoperiod (Table 1).

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Experimental setup

In this experiment, a total of 10 trees (5 ON and OFF trees) were studied. More specifically, during each period, two trees in ON conditions were considered. Only one OFF tree was considered in all periods except the fourth period, for which two trees were used (Supplementary Material Table S2). For ON trees, in 2016, three trees were followed throughout the summer (periods 1, 2 and 3). In 2017 (period 4), two other trees were sampled. These trees differed from those used in 2016 because ON trees in 2016 became OFF trees in 2017 due to biennial bearing. The crop load of each tree was estimated as the ratio of the fruit number to the trunk cross sectional area estimated in autumn. The ON trees displayed high crop loads (14.2 and 18.0 fruits cm^{-2} in 2016 and 2017, respectively, Supplementary Material Table S2). Conversely, OFF trees had a crop load of 0.

All studied trees were equipped with autoleveling devices for measuring locally transmitted PAR (PAR_T). In each period, 18 and 12 PAR devices were distributed within the crowns of ON and OFF trees, respectively. In total, 120 microclimate conditions were recorded during the experiment. For the ON trees, six shoots were chosen in sunlight (outer crown), six other shoots were located in the middle crown, and the final six shoots were located in shade (inner crown). For these trees, sensors were placed close to fruiting (three sensors per light condition) and nonfruiting shoots (three sensors per light condition). For the OFF trees, the same design was used for contrasting light conditions but with only four shoots per light condition. On all the trees, the devices were placed close to short (spurs, length <5 cm) and medium shoots (elongated shoots, length >5 cm).

The PAR sensors (PAR/CBE 80, Solems, Palaiseau, France) were mounted in aluminum bodies housing batteries and an electronic card dedicated to data storage and wireless communication. These homemade devices were placed on a gyroscopic mount fixed to an aluminum mast inserted into the soil. This setup enabled horizontal autoleveling for correct radiation measurements at any location within the crown. The sensors measured local PAR_T every minute and stored 15-minute averages in the internal memory. Prior to each vegetation season, all sensors were calibrated against a Licor 190R Quantum Sensor for 24 hours for one-minute time steps ($R^2 > 0.99$, data not shown). The PAR sensors were placed less than 2 cm from the leafy shoots.

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Leaf gas exchanges and chlorophyll measurements

At the end of each period, just after removing the *PAR* sensors, the leaf with the largest surface area on each monitored shoot was chosen for gas exchange measurements (n = 120). Measurements were performed with a Licor 6400 system (Licor, Lincoln, USA) for two to three consecutive days in each period. Measurements were performed under nonlimiting climatic conditions in a measurement chamber (*PAR* = 1800 µmol m^{-2} s^{-1}; leaf temperature (T_{leaf}) = 30 °C; vapor pressure deficit = 2 kPa; air CO_2 concentration= 390 ppmv; Massonnet *et al*. 2007). After 5-10 min of stabilization, the net CO_2 assimilation (A_{net}) was recorded during measurement sessions lasting from 7:00 AM to 12:00 AM (GMT+2). After this period, the air temperature was too high to maintain optimal conditions in the cuvette.

Leaf sampling and biochemical analyses

The shoots for which leaf gas exchange was measured were sampled. We measured the length (*L*) and width (*W*) of the leaf on which gas exchange measurements were performed. Its area (*A*) was then estimated using an allometric relationship built on the trees used in this study (*A*= 0.73 × *L* × *W*, R²=0.92, n=320). A proxy for chlorophyll content was measured by a SPAD-502 chlorophyll meter (Konica-Minolta, Tokyo, Japan). Then, these leaves were frozen in liquid N and freeze-dried, dry-weighed and finally ground prior to biochemical analyses. The main sugar and starch contents were determined after ethanol extraction by high-performance liquid chromatography (HPLC) and spectrophotometry after enzymatic digestion, respectively (Charrier and Améglio, 2011; Quentin *et al*., 2015). Concentrations of *NSC* were computed from daily calibration curves and expressed as a % of the dry matter. The total N concentration (in % of dry matter) was assessed using a CHN analyzer (EA/NA 1110, Thermoquest). In total, 120 leaves were analyzed for NSC and N contents.

The lengths of a subset of shoots (n = 86) were also measured for ON and OFF trees. Shoot leaf number was estimated from shoot length assuming a linear relationship (shoot leaf number = 0.42 × shoot length + 4.51, R²=0.65, n = 108).

Data processing and analyses

The *LMA* was computed from the *LDW* and *La*. Leaf N content per area unit (*Na*) was obtained by multiplying the *LMA* by the N concentration (*Nm*). SPAD readings were converted into chlorophyll content per area unit (*Chl*) using a consensus equation published by Cerovic *et al*. (2012), and the chlorophyll proportion per *Na* (*Chl:Na*) was computed.

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Mean daily PAR_T and incident PAR (PAR_i) were calculated as the average values over the last 15 days before photosynthesis measurements and sampling. Leaves were separated into three classes depending on the actual amount of intercepted radiation. In each period, the ten leaves with the highest PAR_T were assigned to the high-light group (H), the ten leaves with the lowest values were assigned to the low-light group (L), and the other ten leaves were assigned to the medium-light group (M).

Statistical analyses were performed with R software (R Core Team, 2017). Three-way ANOVA considering light conditions (high, medium, low), crop status (ON, OFF), periods of measurements (accounting for both possible temporal and tree effects) and an interaction between light conditions and bearing status were performed for the A_{net}, Na, Chl, $Chl:Na$, and NSC contents. These analyses were followed by Tukey-HSD tests for pairwise comparison considering all the crop status-light combinations. Two-way ANOVA was used to analyze the consistency of light condition and crop load effects among periods by considering each period separately. The impact of local fruit on these variables was analyzed by comparing leaves belonging to fruiting and nonfruiting shoots on ON trees by two-way ANOVA considering shoot type and period effects. To estimate the effect on individual trees for each crop status, two-way ANOVAs considering tree and light conditions were performed on each date. The effect of the type of shoots (spur or elongated shoots) was tested, and no major significant differences were detected (data not shown).

Correlation analyses were conducted to assess the relationships between A_{net}, Na and NSC concentration, between the subvariables involved in Na variation (LMA, LDW, Nm, La) and between all NSC forms. These correlation analyses were performed considering the whole dataset or ON and OFF trees separately.

Structural equation modeling (path analysis, Shipley 2016) was performed to analyze the relationships between NSC forms and A_{net}. Previous studies (e.g., Tisné *et al*., 2008; Shipley, 2013) have sought the best models in terms of performance and parsimony, testing different sets of variables and structures. In our study, we chose to fit the same model structure employing the same variables to demonstrate the effects of crop status on the coefficients. This approach considers covariations among variables for computing these coefficients, in contrast to usual correlation matrix approaches. We used a model assuming (i) causal relationships between A_{net} and each of the reserve and transported NSCs, i.e., starch, sorbitol and sucrose, and (ii) causal relationships between each of the reserve and transported

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*NSC*s and hexoses (i.e., mainly glucose and fructose), and (iii) free correlations (covariations) between starch, sorbitol and sucrose and between fructose and glucose. This analysis was performed with the Lavaan package of R.

RESULTS

Light variation with treatments and testing period

In comparison to its value under high-light conditions, the average daily PAR_T was reduced by approximately one-third and two-thirds in medium- and low-light conditions, respectively, regardless of the testing period (Table 1). In 2016, the highest PAR_T values were observed in periods 1 and 2, while they were slightly lower in period 3, likely due to the shorter photoperiod. In 2016, PAR_T in high-light conditions represented more than 60% of the $PARi$, while it represented between 15 and 30% in low light conditions. In 2017 (period 4), PAR_T values were lower than those in 2016 for all light conditions. PAR_T represented 32% and 13% of the incoming PAR in high- and low-light conditions, respectively. This reduction in the proportion of PAR_T compared with that of the periods in 2016 was likely associated with (1) a lack of pruning in winter 2016-2017, thus leading to greater self-shading within the canopy and (2) a deeper location of the sensors in the canopy in 2017. Finally, no major difference in PAR_T was observed between the two crop statuses (ON and OFF trees). Only a slight but significant difference (P=0.032) was observed under high-light conditions due to the higher PAR_T for OFF trees.

Effect of crop status and light on carbon- and nitrogen-related variables

A_{net} was strongly affected by the crop status, with greater values for ON trees than for OFF trees regardless of the local light conditions (Fig. 1A, mean values = 13.2 and 7.6 µmol m^{-2} s^{-1} for ON and OFF trees, respectively). The impact of light was lower but still significant. These differences were associated with a significant decrease in A_{net} for low-light conditions. The effect of light was significant for ON trees only. Nevertheless, the interaction between crop status and light conditions was statistically insignificant, meaning that this general trend of A_{net} variation with light intensity was similar regardless of the crop status. A significant period effect was observed, but the effect of the crop status and light condition remained similar if each measurement period was considered separately (Supplementary Fig. S1). No significant individual tree effect on A_{net} was observed in any period (Supplementary Fig. S1).

Moreover, A_{net} was similar for fruiting and nonfruiting shoots of ON trees (Fig. 1B), showing the absence of any effect of local fruit.

Na was significantly lower in OFF trees than ON trees (Fig. 2). This difference was quite small (1.79 and 1.59 g m^{-2} for ON and OFF trees) and was significant in period 1, but only if the analysis was performed for each period separately (Supplementary Fig. S2). Light also had a significant effect on Na. This effect was associated with lower values in ON trees under low light (Fig. 2). If the four periods were considered separately, these significantly lower values were observed in periods 1 and 2 (Supplementary Fig. S2). Conversely, the decreased Na under low-light conditions was not observed for OFF trees, leading to a significant crop status × light effect. For ON trees, local fruit had no effect on Na, consistent with the results for A_{net} (Fig. 2A). Furthermore, as for A_{net}, no significant individual tree effect on Na was observed regardless of the period (Supplementary Fig. S2).

Chlorophyll contents (*Chl*) were higher for ON trees than for OFF trees (0.55 *vs* 0.36 g m^{-2}, Fig. 3A) regardless of the light. These higher values resulted from higher Na values but also from a significantly higher proportion of *Chl* per Na (*Chl:Na*) for ON trees (26.3 and 23.3% for ON and OFF trees, respectively, P<0.01, Fig. 3B). The effect of crop status on *Chl* was significant in all periods, while crop status affected *Chl:Na* in period 4 only (Supplementary Figs. 3 and 4). This increase in *Chl:Na* for ON trees was significant under only low light. A slight but significant effect of light was also observed for *Chl* and *Chl:Na*. Specifically, *Chl* had greater values under medium light than under low light, while *Chl:Na* had higher values under low light in ON trees only. The effects of light were observed in periods 1 and 3 for *Chl* and *Chl:Na*, respectively. Once again, the individual tree effect remained low and was significant for *Chl* in ON trees during period 1 only (Supplementary Figs. S3 and S4).

The crop status significantly affected starch, sorbitol and sucrose concentrations but in different ways (Fig. 4). Indeed, starch and sorbitol concentrations increased in OFF trees compared with ON trees, while sucrose concentrations slightly decreased. Moreover, the impact of crop status was greater on starch (+78% for OFF trees) than sorbitol (+19%). These effects of crop status on starch, sorbitol and sucrose concentrations were observed in almost all periods if considered separately (Supplementary Figs. S5, S6 and S7). The impact of light on *NSC* concentrations appeared lower. Light did not modify sorbitol concentration and slightly but significantly affected starch and sucrose concentrations, with a decrease under low light. These effects of light were found for almost all the measurement periods. Finally, the interaction between crop status and light was not significant for these three *NSC* forms.

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The effect of light was observed in some periods only (Supplementary Figs. S5, S6, and S7). Again, individual effects were not significant for these *NSC* forms. Regarding glucose and fructose concentrations, no significant crop status, light or interaction effects were observed (Supplementary Fig. S8). For A_{net} and Na, *NSC* concentrations were similar between fruiting and nonfruiting shoots of ON trees (Supplementary Fig. S9).

Analysis of relationships between photosynthesis, light harvesting traits and *NSC* contents

When the datasets from both crop statuses were considered together, a negative correlation between A_{net} and starch was observed (Fig. 5A). Nevertheless, this relationship was not significant for OFF trees. In contrast, the relationship was significant and positive for ON trees. Na and A_{net} were positively but slightly correlated when datasets for both crop statuses were considered together and for ON trees. This relationship was not significant when OFF trees were considered separately (Fig. 5B).

Crop status affected all the variables explaining variations in Na (*LDW*, *LMA*, Nm and La) (Fig. 6) but had an opposite effect on Na. Indeed, slightly lower La (which increased *LMA* and Na) and *LDW* (which decreased *LMA* and Na) values were observed for ON trees. Nevertheless, the crop status had a smaller effect on La (-14%) than on *LDW* (-32%), resulting in lower *LMA* for ON trees than for OFF trees (Fig. 6B, 93.5 and 81.1 g cm^{-2} for OFF and ON trees, respectively). Similarly, lower *LMA* (decreasing Na) and higher Nm (increasing Na) were observed for ON trees than for OFF trees. Crop status had a smaller effect on *LMA* (-17%) than on Nm (+30%), consistent with a slightly higher Na for ON trees (Fig. 2). The crop status also affected the mean number of leaves per shoot, with greater values for OFF trees than for ON trees (8.2 *vs* 5.7 leaves, data not shown).

Regarding correlations with PAR_T, large variability was observed, but it depended on the variables and the crop status (Fig. 6). If data from both crop statuses were considered together, PAR_T was not significantly correlated with La or Nm concentration but was significantly positively correlated with *LMA* and *LDW*. Moreover, PAR_T was significantly correlated with *LDW*, *LMA* and Na concentration for ON trees, while a small significant correlation was found with *LMA* for only OFF trees.

Relationships between *NSC* forms and A_{net} strongly depended on the crop status (ON *vs* OFF trees, Fig. 7). First, A_{net} was strongly positively associated with sucrose and starch concentrations in ON trees, whereas the path coefficients related to A_{net} were only slightly significant (P=0.036) with sucrose in OFF trees. In ON trees, the starch concentration

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covaried with sucrose and sorbitol, but this result was not observed in OFF trees. Conversely, for OFF trees, sorbitol and sucrose covaried significantly. For ON trees, the impact of sucrose concentration on hexoses was quite low, and sorbitol and starch concentrations had no impact. For OFF trees, sorbitol, sucrose and starch concentration influenced (P<0.001) the hexose concentrations. The correlations were positive between sorbitol, starch and hexoses and negative between sucrose and hexoses. Finally, for both crop statuses, glucose and fructose concentrations covaried. Correlation analyses (Table 2) confirmed most of the relationships observed in the path analysis. Nevertheless, for ON trees, the relationships between starch and the other *NSC*s (sucrose, starch) observed in the path analysis were not observed in the correlation analysis. Similarly, for OFF trees, the relationships between sucrose and glucose and between sorbitol and fructose highlighted by the path analysis were no longer observed in the correlation matrix. This phenomenon likely resulted from the structure of the path analyses, which accounted for partial correlations between the *NSC* forms.

DISCUSSION

A_{net} is not determined by the local fruit on highly fruited trees

Previous results for apple trees have shown that fruit proximity can affect photosynthesis on branches subjected to different girdling practices (Poirier-Pocovi *et al*. 2018). In mango, Urban *et al*. (2003) also observed an effect of fruit presence on nearby leaf photosynthesis due to changes in *Na*. In our study, local fruit presence had no effect regardless of the measured variables, consistent with results other studies (Belhassine *et al*. 2019). Such a discrepancy is likely associated with the very high crop loads under our conditions (more than 10 fruit cm^{-2}) compared with those under usual orchard management (Wünsche *et al*. 2000). Under high crop load, maximal investment in C assimilation and rapid export of photoassimilates from all leaves are required to sustain fruit growth at the tree scale, thus explaining the lack of differences between leaves. Thus, our results suggest a sink strength above which the global C demand requires maximizing A_{net} at the canopy scale. This maximization can be achieved by optimizing both light capture and C assimilation, and by modifying C allocation as observed in this study and further discussed.

Effect of crop load status on photosynthesis optimization by acclimation of leaf N-related variables within trees

Different leaf traits are key variables for describing leaf C function and acclimation to light gradients across a various range of plant species and are part of the so-called leaf economics spectrum (Wright *et al*., 2004; Niinements *et al*., 2015, Onoda *et al*., 2017). Nevertheless, the combined effects of crop load and light were not investigated in previous studies. Our study thus challenges the general applicability of that framework for systems with two contrasting source-sink relationships.

Several studies reported enhanced A_{net} in ON trees compared with OFF trees (as shown by Wunsche *et al*. 2000 on apple, Vaast *et al*. 2005 on coffee, and Wu *et al*. 2008 on peach). Enhancing A_{net} implies that leaves need more energy for both photochemical and biochemical processes of photosynthesis. At the leaf level, enhancing light harvesting and use can be achieved by increasing light absorption efficiency through increasing the content of light-absorbing molecules such as chlorophyll. Among the covariables that could explain the increase in A_{net} depending on the crop status, Na values were higher in ON trees than OFF trees. The apparent effect of the crop status (ON *vs* OFF trees) on Na might be attributed to variations in Nm. Lower Nm values have been reported for trees with low crop loads than for trees with high crop loads (Stander *et al*. (2017) for mandarin; Syvertsen *et al*. (2003) for orange; and Urban *et al*. (2004) for mango). If we consider ON trees only, our results are in agreement with those of these previous studies and consistent with the optimization hypothesis (Hollinger *et al*. 1996, Thornley, 1998, 2004). Nevertheless, one original aspect of our study is that such an impact of light was poorly observed for OFF trees despite the strong PAR_T gradient. Our results thus suggest that, under the growing conditions of the study, optimal allocation of N within trees to maximize light harvesting and photosynthesis occurs only above a certain fruit C demand. This implies that trees adjust N absorption and further N allocation to leaves depending on the crop load (Ding *et al*., 2017). For a better understanding of the impact of C demand on plant C and N functions (Paul and Foyer, 2001), N partitioning into light harvesting, bioenergetics and biochemistry pools might require analysis. This partitioning has been documented based on the model of Niinemets and Tenhnunen (1997) for different plant functional types (see the review of Niinemets *et al*. 2015 for nonfruiting species, Frak *et al*. 2006 on walnut, and Urban *et al*. 2003 on mango). In that framework, our results reveal a higher concentration of *Chl* in high cropping trees (as reported by Wünsche *et al*. 2005) and a higher proportion of *Chl* in *Na* in shaded leaves (as reported by Niinemets *et*

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al. 2004). Our study thus suggests that high fruit C demand might promote optimization of A_{net} by increasing N investment in the light harvesting pool in response to a light gradient.

Effect of crop load status on photosynthesis optimization by acclimation of leaf structural characteristics within trees

Leaf structural traits such as *LMA* are central players in the leaf economics spectrum (Wright *et al*., 2004; Niinements *et al*., 2015, Onoda *et al*., 2017), but the combined effects of crop load and light on these variables have been poorly investigated. In our study, significant relationships between PAR_T and *LMA* were found for ON trees only, and mean *LMA* values were lower in ON trees. Few studies have investigated the impact of crop load on *LMA* (Schechter *et al*., 1994; Urban *et al*., 2004), but they did not evaluate the variation in *La* and *LDW*, which are used to calculate *LMA,* as performed in this study. Our results show that the relationship between PAR_T and *LMA* for ON trees was related to variations in *LDW*. The decrease in *LMA* due to crop load could also be attributed to a stronger decrease in *LDW* than in *La* for ON trees. A smaller investment in leaf dry matter in ON trees could be an adaptive trade-off to achieve satisfactory light interception efficiency, i.e., a minimal amount of biomass is invested when the fruit C demand is high on the plant scale. In parallel, *LMA* can also be considered the product of leaf thickness and leaf density; higher thickness and lower density could result in higher A_{net} due to higher photosynthetic compound content and greater internal CO_2 diffusion to chloroplasts, respectively (Niinemets 1999). Hence, in the present study, the increased A_{net} in ON trees coupled with lower *LMA* in ON trees was likely to be associated with lower density.

Consistent with other studies in fruit trees (e.g., Palmer *et al*. 1992 and Inglese *et al*., 2002), a negative relationship between vegetative primary growth and crop load was observed as the outcome of a high level of competition for C between vegetative and reproductive growth. This lower C allocation to the vegetative compartment can result in decreased light interception in high cropping trees (Wünsche *et al*., 2000). At the tree scale, the carbon demand of fruits in ON trees having this decrease in light interception could be sustained by improved radiation use efficiency through increased *Nm* and chlorophyll content and optimization of the N allocation to sunny leaves (Zanotelli *et al*., 2016).

***NSC* interplay within trees as affected by crop load status from a sink-driven perspective**

Several studies have reported the impact of light gradients on the spatial variability of *NSC*s (Lichtenthaler *et al*., 1981; Le Roux *et al*., 1999). In contrast, our results showed nearly no effect of light on the different *NSC* concentrations. These results suggest similar C statuses and/or export rates across the crown despite significantly different A_{net} values. Such a result is likely related to the homogeneous distribution of the fruit within the trees for both crop statuses in our study, resulting in a homogeneous distribution of the leaf export rate.

Regulation of photosynthesis by the fruit or seed C demand has been previously observed, and two main mechanisms, i.e., stomatal closure and end-product saturation, have been discussed (Andrade *et al*., 2019; Gucci *et al*., 1995; Wünsche *et al*., 2005). In our study, the most marked effect was the strong depletion in leaf starch for high crop load regardless of the light (Naschitz *et al*., 2010; Quilot *et al*., 2004). In addition, the difference in the relationships between A_{net} and starch content highlights differences in C function between the two crop statuses. In ON trees, the synthesized photosynthates are rapidly exported to sustain fruit growth (Breen *et al*., 2020; Génard *et al*., 2003), with C even being imported from nonbearing shoots (Walcroft *et al*., 2004), thus leaving a minimal amount of starch in leaves. The positive correlation observed in ON trees between A_{net} and the starch content was more surprising. The positive correlation can be interpreted as resulting from the starch pool that is likely refilled proportionally to both the source capacity and fruit importation rate, advocating for it being considered a priority sink under high crop load, which is very scarcely observed in trees (Lescouret *et al*., 1998; Silpi *et al*., 2007). Conversely, in OFF trees, the photosynthates are oriented towards only vegetative growth. In these trees, the leaf starch pool was filled due to low C demand at the tree scale. This starch pool was likely dedicated to reserves for sustaining further organ primary and/or secondary vegetative growth (root, shoots, and branches). Finally, this starch accumulation might downregulate A_{net} (Nebauer *et al*., 2011; Poirier-Pocovi *et al*., 2018; Urban *et al*. 2004; 2007), thus canceling its spatial variability.

Similar to starch, light had nearly no effect on leaf sucrose and hexose contents. Nevertheless, sorbitol content was lower in ON trees than OFF trees, suggesting a higher use rate for fueling fruit growth (Pallas *et al*., 2018). Moreover, the relationships among the *NSC* and A_{net} differed between the ON and OFF trees, as shown by the path and correlation analyses, revealing different C allocation patterns for the two crop loads. In ON trees, the pattern that could be drawn was a major allocation of photosynthates to transport to outside

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the leaf, especially for sucrose and, to a lesser extent, sorbitol. The relationships among sucrose, glucose and fructose were also consistent with the known pathway of sucrose biosynthesis. The relationships between A_{net} and starch and among starch, sorbitol and sucrose reveal the central role of the latter group as an intermediary pool directly fueled by photosynthesis. In contrast, in OFF trees, the path model showed a feedback loop between sucrose and sorbitol, with the latter being related to both glucose and fructose, which in turn were related to sucrose. Such cyclic interactions might reflect other roles of sucrose and sorbitol as temporary storage forms when the leaf starch pool has likely reached high levels for further use in vegetative growth or storage (Escobar-Gutierrez and Gaudillère, 1996; Genard *et al.*, 2003).

CONCLUSIONS

Although previous studies have investigated the impact of light microclimate and fruit load on leaf traits (functional and structural), none of them have analyzed their combined effects. In this context, our study reports original results and demonstrates that crop status has a greater influence than local light or local fruit on all the measured traits. Moreover, our study clearly shows that the intensity of leaf acclimation to light strongly depends on the carbon demand at the tree scale. Indeed, acclimation of photosynthesis to light was mainly observed for ON trees, which was achieved mainly by increased light capture efficiency, implying higher leaf N and lower *LMA*. Conversely, low C demand, as in OFF trees, is thought to disrupt the optimal distribution of resources by modifying the interplay among *NSC*s. These experimental results have potential implications for shifting modeling approaches towards a more mechanistic representation of optimal strategies between C and N within-tree allocations depending on the crop load. Our study also supports a consistent description of the possible role of starch in regulating photosynthetic activity and its spatial variability according to crop load. Under high crop loads, starch could be one of the priority sinks. Under high crop loads, high starch levels promote the optimization of photosynthesis and related traits, while under low crop load, high starch levels tend to downregulate photosynthetic activity. Moreover, sorbitol and sucrose play major roles in the transport of carbohydrates when fruit demand is high, while they serve as temporary reserves under low crop loads in apple trees.

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DATA AVAILABILITY

The data that support the findings of this study are openly available in a Data INRAE repository (doi: XXXXXX to be completed).

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

JN and BP designed the experiment. AM and JN installed and monitored the functioning of the *PAR* sensors. SM, BP and SB performed the field measurements, and BSJ and SB performed the biochemical analyses. BP and JN performed the statistical analyses and wrote the manuscript. EC reviewed and improved the manuscript.

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TABLES

Table 1. Mean values of daily incoming photosynthetically active radiation (*PARi*, +/- standard deviation among days) and mean values of transmitted PAR for the two crop statuses ('ON' and 'OFF') and the three light environments in the four measurement periods (+/- standard deviation among leaves of each light/crop load environments).

Periods	Starting - Ending dates	Crop status	Incoming PAR (PAR_i, $mol.m^{-2}.day^{-1}$)	Transmitted PAR (PAR_T, $mol.m^{-2}.s^{-1}$) High Light (H)	Medium Light (M)	Low Light (L)	*Light condition*	*Crop Status*
1	26 June - 10 July 2016	'ON'	53.7 +/- 8.4	35.4 +/- 6.2 (3)	23.8 +/- 5.0 (9)	13.3 +/- 3.9 (6)	***	ns
		'OFF'		35.8 +/- 5.4 (7)	20.7 +/- 0.0 (1)	15.9 +/- 1.6 (4)		
2	15 July - 01 August 2016	'ON'	46.5 +/- 9.8	33.2 +/- 2.9 (6)	21.8 +/- 2.9 (7)	11.3 +/- 3.8 (5)	***	*
		'OFF'		37.5 +/- 3.4 (4)	25.5 +/- 1.4 (3)	13.9 +/- 1.9 (5)		
3	17 August - 31 August 2016	'ON'	43.3 +/-8.6	26.2 +/- 1.9 (4)	16.0 +/- 3.9 (5)	8.7 +/- 3.1 (9)	***	ns
		'OFF'		27.3 +/- 5.0 (6)	17.6 +/- 3.7 (5)	6.8 +/- 0.0 (1)		
4	11 July - 25 July 2017	'ON'	51.6 +/-8.9	16.0 +/- 3.6 (6)	11.0 +/- 0.9 (7)	6.3 +/- 2.8 (5)	***	ns
		'OFF'		16.5 +/- 4.4 (4)	11.8 +/- 0.8 (3)	7.8 +/- 1.9 (5)		
		Date		***	***	***		
		Crop status		*	ns	ns		

Two-way ANOVA were performed for the PAR_T values considering first each light condition separately for the four periods and the two crop status, and second each measurement period separately for the three light conditions and the two crop status

Table 2. Correlation coefficient and associated level of significance between the non-structural carbohydrates forms and net carbon assimilation (A_{net}). The upper and lower panels correspond for 'OFF' and 'ON' trees, respectively.

	Glucose	Fructose	Sucrose	Sorbitol	Starch	A_{net}	
Glucose		**0.78*****	ns	**0.56*****	0.36*	ns	
Fructose	**0.92*****		-0.48**	ns	0.35*	ns	**'OFF'**
Sucrose	-0.30**	-0.29*		**0.59*****	ns	0.35*	**trees**
Sorbitol	ns	ns	ns		ns	ns	
Starch	ns	ns	ns	ns		ns	
A_{net}	ns	-0.27*	**0.75*****	ns	**0.61*****		

'ON' trees

*** significant at P<0.001, ** significant at 0.001<P<0.01, * significant at 0.01<P<0.05, ns non significant.*

FIGURE LEGENDS

Figure 1. A. Boxplot representation of net carbon assimilation (A_{net}) for the two crop statuses ('ON' and 'OFF') and the three light conditions (H: High, M: Medium, L: Low) considering together the four measurement dates. B. Boxplot representation of A_{net} in the fruiting and non-fruiting shoots in 'ON' trees considering together the four measurement dates. In A, a three-way ANOVA with crop status, light condition and period effects, and the light condition × crop status interaction was performed. This analysis was followed by a pair-wise Tukey test considering all the crop status-light condition combinations and letters indicate significant differences among groups at P<0.05. In B, a two-way ANOVA with fruit presence and period effects was performed. *** significant at P<0.001, ** significant at 0.001<P<0.01 and ns non-significant.

Figure 2. A. Boxplot representation of leaf nitrogen concentration per area unit (Na) for the two crop statuses ('ON' and 'OFF') and the three light conditions (H: High, M: Medium, L: Low) considering together the four measurement dates. B. Boxplot representation of Na in the fruiting and non-fruiting shoots in 'ON' trees considering together the four measurement dates. In A, a three-way ANOVA with crop status, light conditions and period effects, and the light condition × crop status interaction was performed. This analysis was followed by a pair-wise Tukey test considering all the crop status-light condition combinations and letters indicate significant differences among groups at P<0.05. In B, a two-way ANOVA with fruit presence and period effects was performed. *** significant at P<0.001, ** significant at 0.001<P<0.01, * significant at 0.05<P<0.01 and ns non-significant.

Figure 3. Boxplot representations of chlorophyll concentration per area unit (Chl) and of chlorophyll proportion per leaf nitrogen area ($Chl:Na$) for the two crop statuses ('ON' and 'OFF') and the three light conditions (H: High, M: Medium, L: Low) in the four measurement periods. For each period, a two-way ANOVA with crop status, light conditions and the interaction between light condition and crop status was performed. *** significant at P<0.001, ** significant at 0.001<P<0.01, * significant at 0.01<P<0.05, and ns non-significant. This analysis was followed by a pair-wise Tukey test considering all the crop status-light condition combinations and letters indicate significant differences among groups at P<0.05.

Figure 4. Boxplot representations of starch (A), sorbitol (B), sucrose (C) concentrations in leaves for the two crop statuses ('ON' and 'OFF') and the three light conditions (H: High, M: Medium, L: Low) considering together the four measurement dates. In A, B and C three-way ANOVA with crop status, light conditions and period effects, and the light condition × crop status interaction was performed. These analyses were followed by pair-wise Tukey tests considering all the crop status-light condition combinations and letters indicate significant differences among groups at P<0.05. *** significant at P<0.001, ** significant at 0.001<P<0.01, * significant at 0.05<P<0.01 and ns non-significant.

Figure 5. Correlations between net carbon assimilation (A_{net}) and starch concentration in leaves (A) and between A_{net} and leaf nitrogen concentration per area unit (Na, B). Dotted lines represent the linear regression considering both crop statuses ('ON' and 'OFF') and blue lines represent the linear regression considering 'ON' trees, only. R² values and the level

of significance of the correlations are represented in the top left corner of each graph. *** significant at P<0.001, ** significant at 0.001<P<0.01 and ns non-significant.

Figure 6. Correlations between locally transmitted photosynthetically active radiation (PAR$_T$) and leaf dry weight (*LDW*, A), leaf mass area (*LMA*, B), nitrogen concentration per mass unit in leaves (*Nm*, C) and individual leaf area (*La*, D). Dotted lines represent the linear regression considering both crop statuses ('ON' and 'OFF'), blue lines represent the linear regression considering ON trees, only and red lines represent the linear regression considering 'OFF' trees, only. R² values and the level of significance of the correlations are represented in each sub-figure legend. *** significant at P<0.001, ** significant at 0.001<P<0.01 and ns non-significant. Dashed red and blue lines represent the mean values observed for 'OFF' and 'ON' trees, respectively. A one-way ANOVA was used to estimate the significance of the crop status effect. The level of significance of the effect of the crop status is represented in the first line of the sub-figures legend.

Figure 7. Results of the path analysis performed on net carbon assimilation (*A$_{net}$*) and nonstructural carbohydrate concentrations in leaves on 'ON' and 'OFF' trees. On these graphs, only the significant relationships of the model are represented. Single-headed arrows represent causal relationships and double-headed arrows represent free correlations. Normalized path coefficients together with their significance level were represented above each arrow. *** significant at P<0.001, ** significant at 0.001<P<0.01, * significant at 0.05<P<0.01.
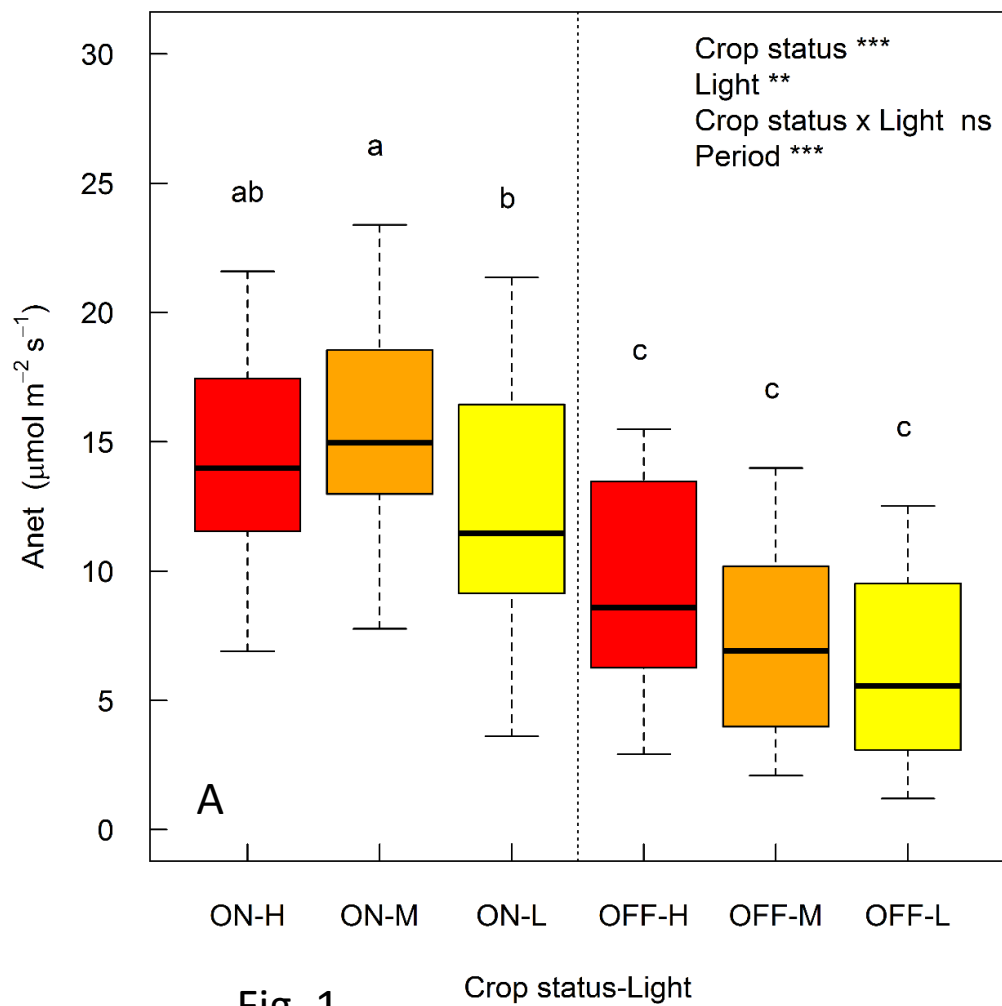
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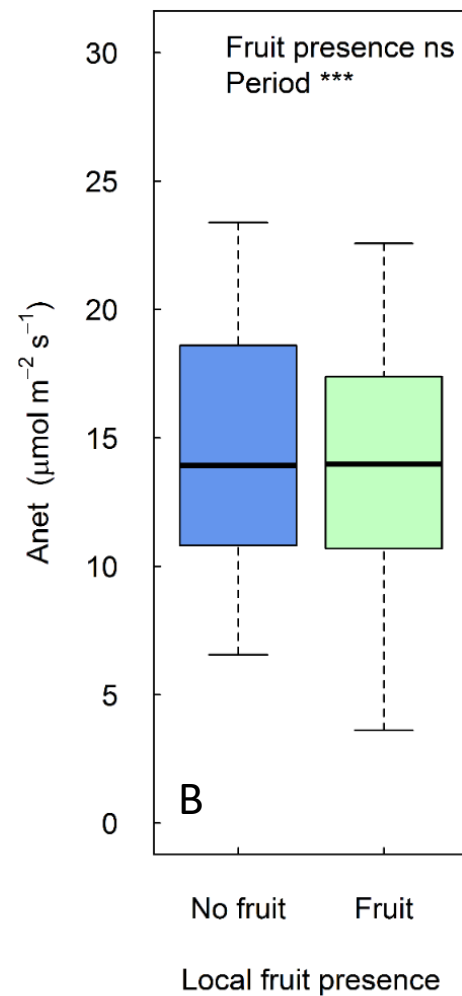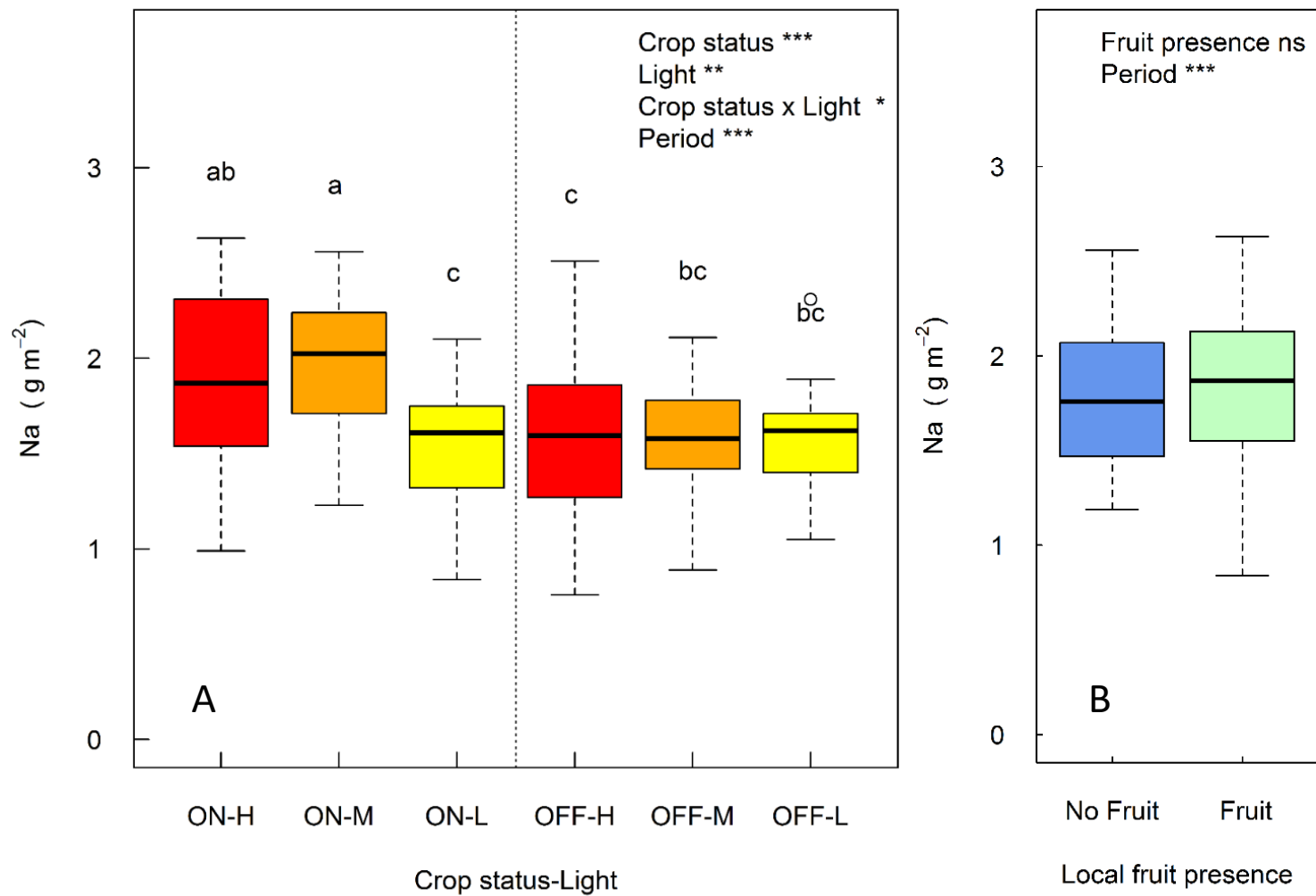
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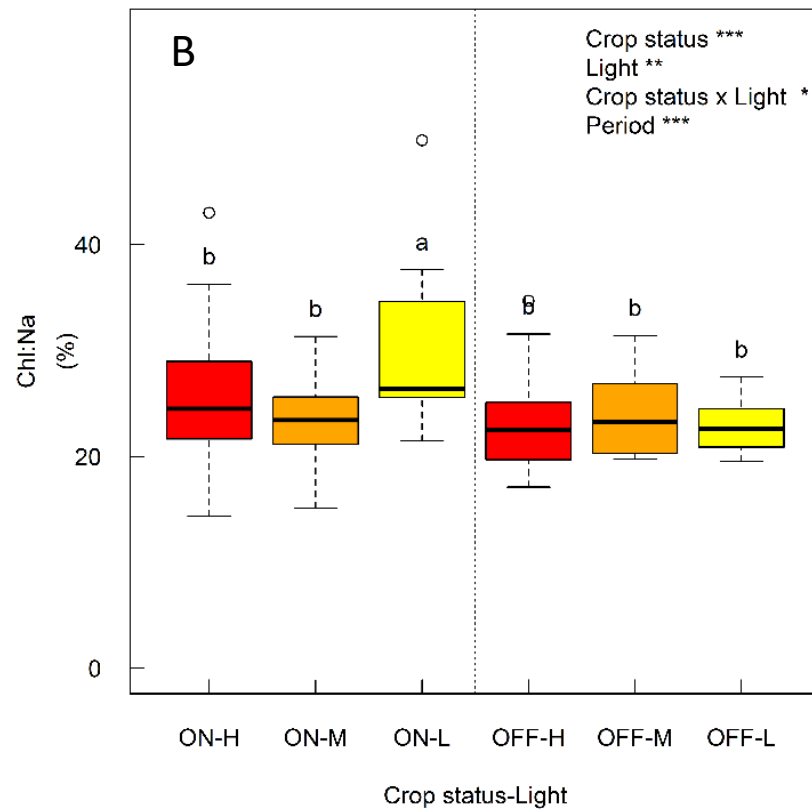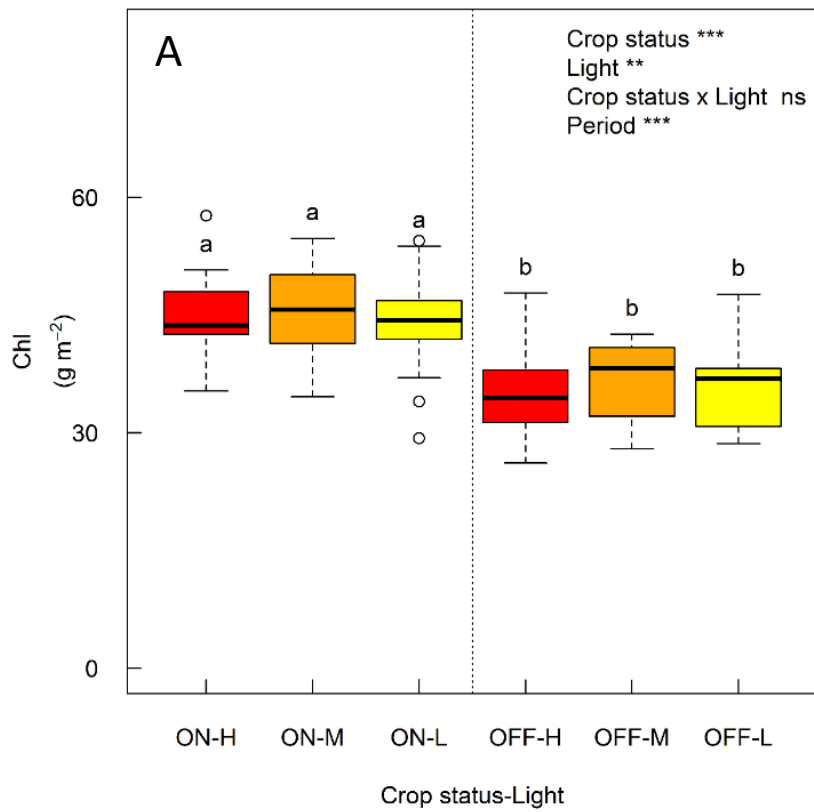
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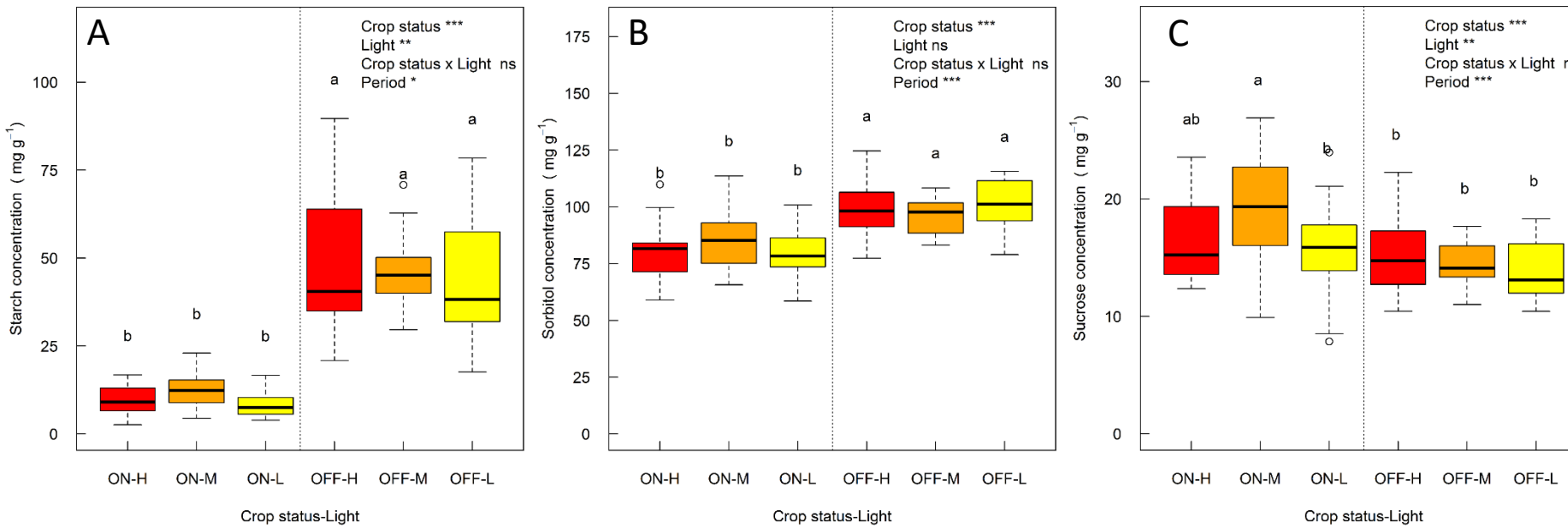
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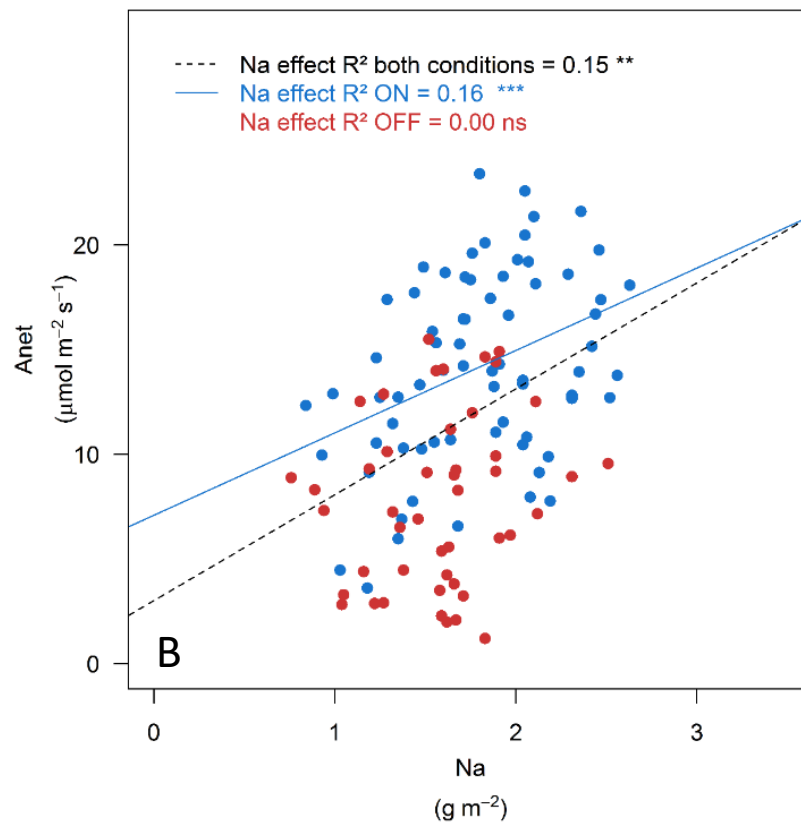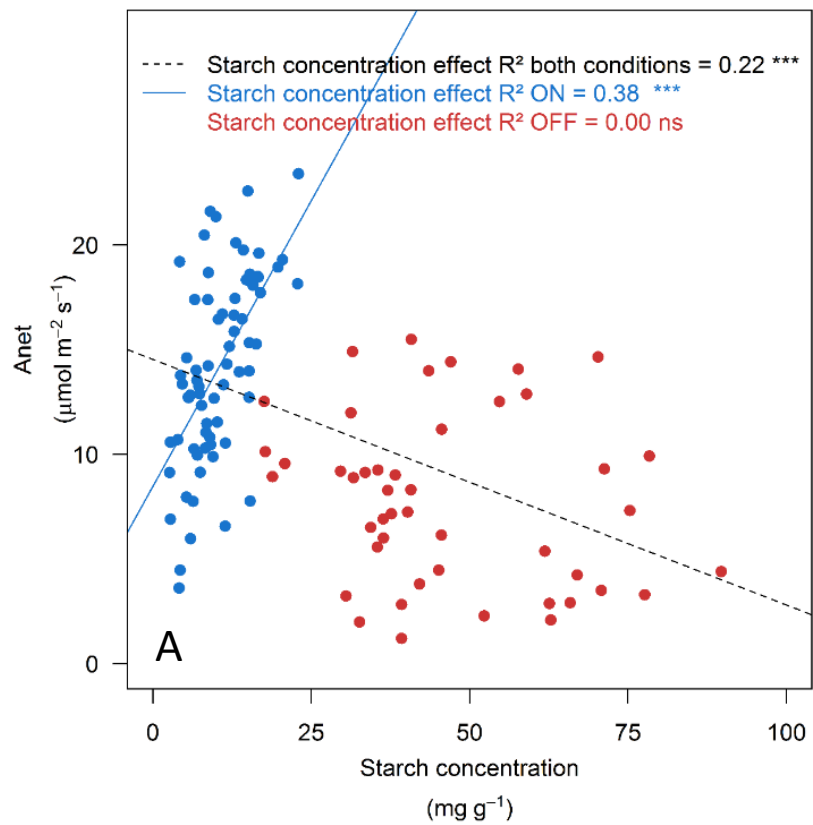
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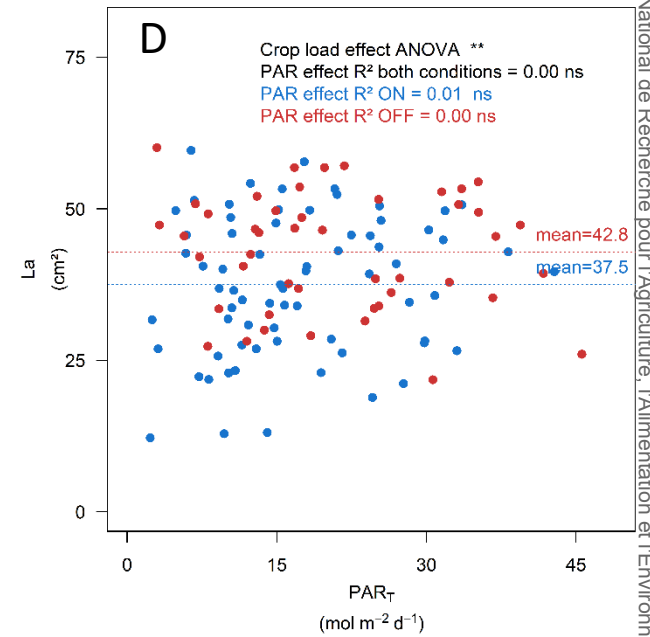
Fig. 6.
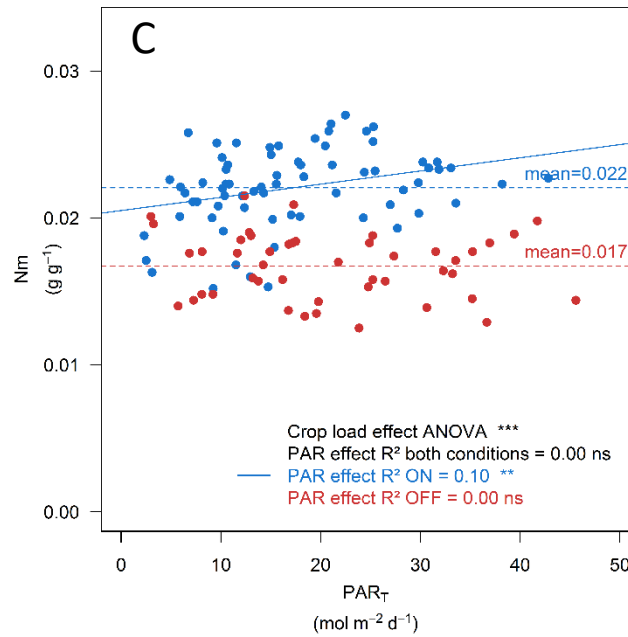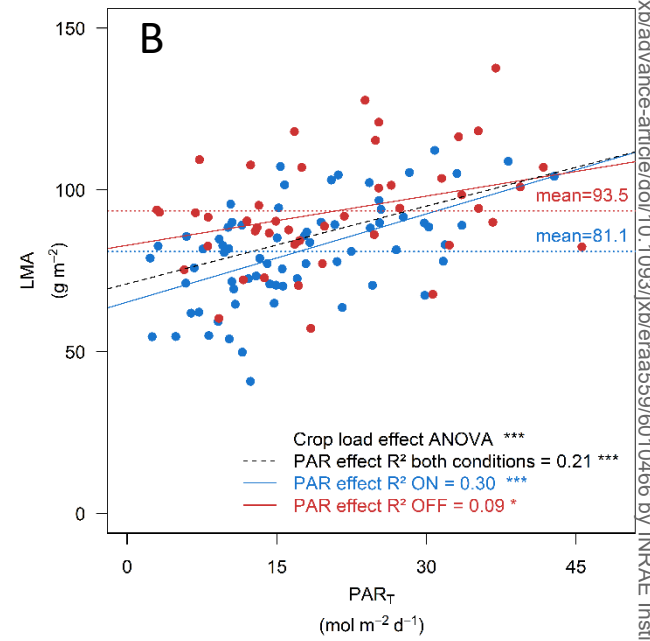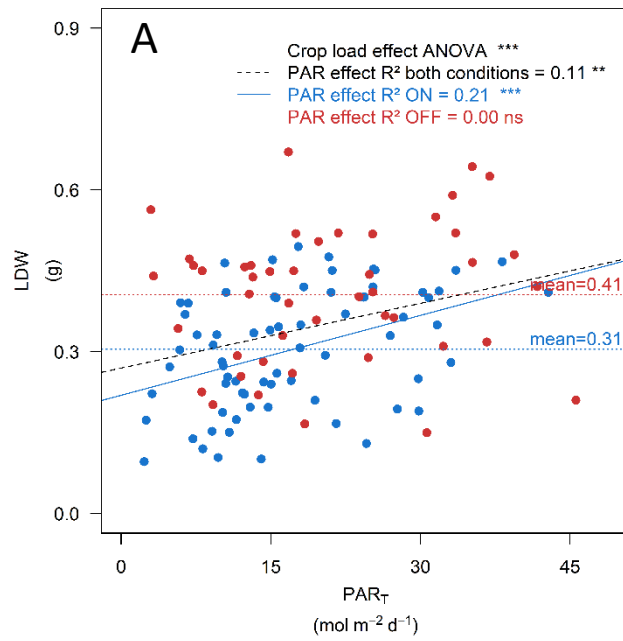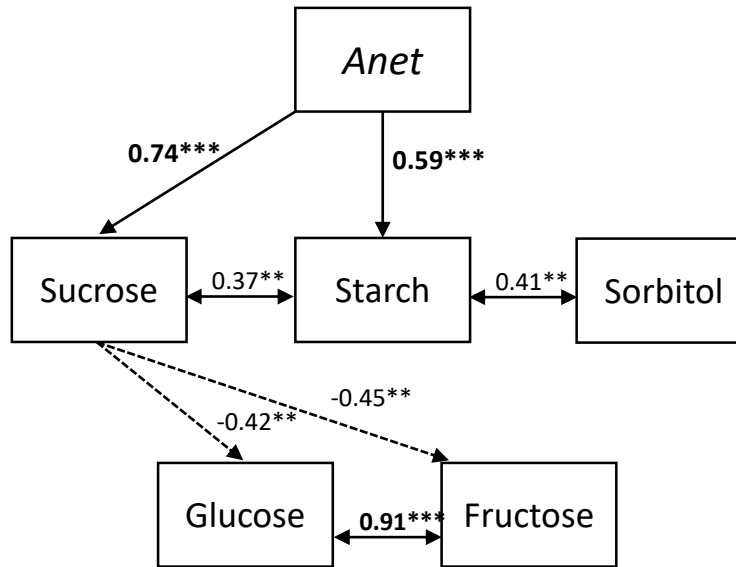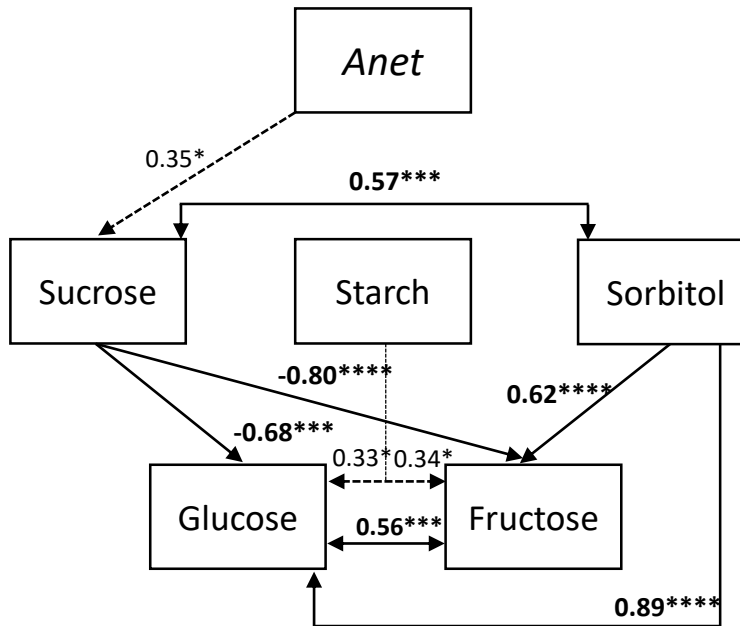
A - 'ON' trees

B - 'OFF' trees

Fig. 7.