UNIVERSITÉ DU QUÉBEC À MONTRÉAL

THE FUNCTIONAL ROLE OF CARBOHYDRATE RESERVES IN THE GROWTH AND SURVIVAL OF TREES

DISSERTATION

PRESENTED

AS PARTIAL REQUIREMENT

OF THE DOCTORATE OF BIOLOGY
BY

JORGE ANDRÉS RAMÍREZ

APRIL 2017

UNIVERSITÉ DU QUÉBEC À MONTRÉAL

LE RÔLE FONCTIONNEL DES LES RÉSERVES DES CARBOHYDRATES DANS LA CROISSANCE ET LA SURVIE DES ARBRE

THÈSE

PRÉSENTÉE

COMME EXIGENCE PARTIELLE

DU DOCTORAT EN BIOLOGIE
PAR

JORGE ANDRÉS RAMIREZ

AVRIL 2017

I dedicate this thesis to my son Emilio, my wife Eliana and, my parents and grandmother Marta, Jorge, and Teresita!

ACKNOWLEDGEMENTS

I would like to thank my advisors, Christian, Tanya, and Juan for their guidance and support during the last five years. I am very proud to have worked with them and, above all, for counting on their friendships. I would also like to thank the rest of my committee members for their help.

Thank you to Björn, Günter, and Dylan for their advice and help all the time. Thanks to Mario, Nathaly, Eugenié, and Lorencito, who made my time at UQAM so wonderful. This study would not have been possible without the help from Milena Molina, Juan Carlos Medina, Luis Carlos Galeano, David Andres Herrera, Juan Guarín, Sergio Martínez, Alvaro Castaño, Manuel Restrepo and Claudia Rivera. I also thank Alfredo Navas (Hacienda Sabaneta Natural Reserve), Alvaro Cogollo (Medellín Botanical Garden), and Martin Gaudet (Montreal municipal nursery) for their logistical support. Thank you to all of the staff from CEF and UQAM for working behind the scenes to make this work successful.

Finally, I would like to thank my son Emilio, my wife Eliana, and my parents and grandmother for being so loving always. I love them very much!

TABLE DES MATIÈRES

LISTE DES FIGURES

LISTE DES TABLEAUX

ABSTRACT

Non-structural carbohydrates (NSC) provide plants with the energy to maintain their metabolism and enhance recovery after a physical disturbance and/or during periods of low photosynthetic activities. Within an individual plant, allocation of carbon to reserve pools is a competitive process between growth and other physiological processes such as defense. Allocation of carbon to NSC in tree tissues depends on the ability of organs to acquire plant-available resources and the physical distance among those organs. Therefore, disturbances that imply loss of tissue will trigger the mobilization of carbohydrates to support compensatory growth and this will affect carbon allocation priorities between growth and reserves. Nevertheless, there are still fundamental unanswered questions about how NSC are stored and used among tissues and among different species. This thesis addressed the global question of how do different levels and types of physical damage affect the recovery of juvenile trees, and how do different tree species used their carbon reserves given their different resource conservation strategies (acquisitive-conservative). Due to the high number of samples I obtained for analysis, and the expenses and time commitment necessary for measuring NSC in plant tissue using regular analytical procedures, I developed a general NSC concentration calibration model using near-infrared spectroscopy (NIRS) in ***Chapter 1***. This model was based on samples from different tree species and tissue types, from tropical and temperate biomes. I obtained parsimonious and accurate calibration models for total NSC and sugars, which demonstrated the ability of the NIRS models to infer NSC concentrations across species and tissues in a rapid and cost-effective way. In ***Chapter 2***, I tested whether there was a coordinated variation between NSC concentrations and the leaf and wood economic spectra. I found that the relationship between functional traits and carbohydrate concentrations was orthogonal. The first axis was formed by traits that define the leaf and wood economics spectrum and the second axis was defined by NSC concentrations.

Additionally, most of the relationships between NSC concentrations in woody tissues and economic traits were weak or non-significant. These results suggest that carbon investment in defense traits that are associated with resource conservation, or 'slow' ecological strategies (high investment in defenses), are not related to investment of carbon in NSC storage. ***In Chapter 3***, I assessed the dynamics of NSC concentrations in different tree parts immediately following a maintenance pruning of the crown of two urban tree species, *Acer saccharinum* and *Acer platanoides*. I found that maintenance pruning did not have any significant depletion effect on carbohydrate concentrations in the different tree parts of either species. On the contrary, NSC concentrations in unpruned branches of pruned trees of *A. platanoides* increased at the end of the growing season, but no effect was measured in A. saccharinum. This differential response suggests that *A. platanoides* responds better to urban maintenance pruning than *A. saccharinum*. This result suggests that maintenance pruning does not impede the capacity of urban trees to produce compensatory growth from accumulated reserve following pruning. ***In Chapter 4***, I evaluated the single and interactive effects of three stress factors (defoliation, root pruning, and stem damage) on NSC concentrations and growth of three tree species that are common urban trees in eastern North America: *Fraxinus pennsylvanica*, *Celtis occidentalis*, and *Tilia cordata*. I found that the predominant effects on NSC concentrations were due to the single stress treatment effects. Also, I found that the effects of single treatments remained after a growing season (more than one year after the last stress application), but that the effects of combined stresses almost disappeared after the first growing season. Additionally, the stress treatments that increased the levels of reserves the most led to a greater decrease in growth of the trees' diameter and height demonstrating the competitive nature between tree reserve and growth. Finally, there were very different responses found among the three tree species investigated and no generalizable trends could be found. These results force a re-examination of the roles

and linkages between NSC in trees and various stress factors that are commonly found in urban environment.

Keywords: Non-structural carbohydrates, tree stresses, compensatory mechanisms, single and multiple stresses, plant carbon allocation strategies, functional traits, tropical forest, temperate forest, urban trees

RÉSUMÉ

Les hydrates de carbone non structuraux (HCNS) fournissent aux plantes l'énergie leur permettant de maintenir leur métabolisme et d'améliorer leur récupération suite à des perturbations physiques et/ou pendant des périodes d'activité photosynthétique plus faible. À l'intérieur d'un individu, l'allocation du carbone dans les réservoirs est conditionnée par un processus de compétition entre la croissance et d'autres processus physiologiques tels que la défense. L'allocation de carbone pour la synthèse des HCNS dans les tissus de l'arbre dépend de la capacité des organes à acquérir des ressources disponibles et de la distance physique entre ces organes. Par conséquent, les perturbations impliquant une perte de biomasse vont déclencher la mobilisation des HCNS afin de compenser la perte de croissance ce qui affecte ainsi les priorités d'allocation de carbone entre la croissance et les réserves. Néanmoins, il y a des questions fondamentales, non encore répondues, sur la façon dont les HCNS sont stockés et utilisés entre les tissus et chez des espèces différentes. La présente thèse aborde la question globale qui est comment les différents niveaux et les différents types de dommage physique affectent-ils la récupération des jeunes arbres. Comment différentes espèces d'arbres utilisent-elles leurs réserves de carbone étant donné leurs différentes stratégies d'utilisation des ressources (acquisition vs conservation) ?

En raison du nombre élevé d'échantillons collectés pour les analyses ainsi que des frais et du temps nécessaire pour mesurer les HCNS dans les tissus végétaux à l'aide des procédures d'analyse classique, j'ai développé, dans le ***Chapitre 1***, un modèle général d'étalonnage de la concentration des HCNS par la spectroscopie en proche infrarouge (NIRS en anglais). Ce modèle était basé sur des échantillons de différentes espèces d'arbres et de différents types de tissus provenant de biomes tropicaux et tempérés. J'ai obtenu des modèles NIRS parcimonieux et précis pour les HCNS ainsi que les sucres, qui ont démontré leur capacité à inférer les concentrations des HCNS à travers différentes espèces et tissus d'une manière rapide et rentable.

Dans le ***Chapitre 2***, j'ai testé s'il y existait une covariation entre les concentrations des HCNS et le spectre économique des feuilles et du bois (traits fonctionnels). Aucune ou de faibles relations entre les traits fonctionnels et les concentrations des hydrates de carbone dans les tissus ligneux ne fut trouvée. Ces résultats suggèrent que les investissements de carbone dans les traits de défense qui sont associés à la conservation des ressources ou à des stratégies écologiques «lentes» (des investissements élevés en défense) ne sont pas liés à l'investissement de carbone dans le stockage des HCNS.

J'ai évalué, dans le ***Chapitre 3***, les dynamiques des concentrations des HCNS sur des parties différentes de l'arbre, immédiatement après un élagage de la couronne chez deux espèces d'arbres urbains: *Acer saccharinum* et *Acer platanoides*. J'ai constaté que l'élagage n'a pas eu d'effet significatif sur l'épuisement des concentrations en HCNS sur les différentes parties des arbres chez les deux espèces étudiées. Au contraire, les concentrations des HCNS dans les branches non élaguées d'arbres chez A. *platanoides* ayant subi un élagage ont augmenté à la fin de la saison de croissance, alors qu'aucun effet n'a été mesuré chez *A. saccharinum*. Cette réponse différente suggère qu'*A. platanoides* répond mieux qu'*A. saccharinum* aux perturbations causés par l'élagage urbain. Ce résultat suggère aussi que l'élagage d'entretien ne limite pas la capacité des arbres urbains à compenser la perte de croissance suite à l'élagage à partir des réserves accumulées.

Dans le ***Chapitre 4***, j'ai étudié les effets propres de trois facteurs de stress (défoliation, taille des racines et endommagement du tronc) ainsi que leurs interactions sur les concentrations des HCNS et sur la croissance des trois espèces d'arbres couramment utilisés en milieu urbain dans l'est de l'Amérique du Nord : *Fraxinus pennsylvanica, Celtis occidentalis et Tilia cordata*. J'ai ainsi trouvé que les principaux effets sur les concentrations des HCNS sont dus aux traitements propres et non aux interactions. En outre, les effets des traitements seuls sont restés après une saison de croissance (plus d'un an après la dernière application du stress) mais que les effets des facteurs de stress combinés ont presque disparu après la première saison de croissance. De plus, les traitements de stress qui ont augmenté le plus les niveaux de réserves ont conduit à une plus grande diminution de la croissance du diamètre et de la hauteur des arbres, démontrant ainsi la compétition qui existe chez les arbres au niveau de leurs réserves et de leur croissance. Finalement, il y a eu des réponses très différentes entre les trois espèces étudiées et aucune tendance généralisable ne fut trouvée. Ces résultats forcent à examiner de nouveau les rôles et les liens entre les HCNS dans les arbres et les divers facteurs de stress généralement observés en milieu urbain.

Mots clés : Hydrates de carbone non structuraux, stress d'arbres, mécanismes de compensation, stress uniques et multiples, stratégies d'allocation de carbone chez les plantes, traits fonctionnels, forêts tropicales, forêts tempérées, arbres urbains.

INTRODUCTION

Trees live under fluctuating and somewhat unpredictable conditions that influence their overall growth and survival strategies. Any sustained deviation beyond the optimum environmental range or normal level of disturbance reduces productivity and constitutes a stress for the plant (Niinemets & Valladares 2006; Niinemets 2010). Additionally, several stress factors may occur in an interactive manner that could generate a response that may be more or less severe than the sum of their individual effects (Mittler 2006; Niinemets 2010). For example, in forest environments, light limitation (Niinemets & Valladares 2006; Myers & Kitajima 2007; Poorter & Kitajima 2007), drought (McDowell *et al.* 2008; Mitchell *et al.* 2013; O'Brien *et al.* 2014), altitudinal limitation at treeline (Handa, Körner & Hättenschwiler 2005; Hoch & Körner 2012; Fajardo & Piper 2014), or herbivory (Kobe 1997; Canham *et al.* 1999; Myers & Kitajima 2007; Atkinson *et al.* 2014) constitute key environmental stresses and disturbances. In urban areas, trees are affected by a multitude of severe abiotic stresses that make the growing conditions even harsher than for trees growing under natural conditions (Sieghardt *et al.* 2005). For instance, many grey infrastructures such as sidewalks, roads and buildings negatively affect tree growth and survival by limiting their growing space, compacting the soil and limiting water infiltration (Sieghardt *et al.* 2005). Human activities that result in soil, water, and atmospheric pollution increase the problem (Konijnendijk & Randrup 2004; Tubby & Webber 2010). Finally, direct damage to the surrounding trees caused by road, sidewalk and building repair and recurrent vandalism further exacerbate the limitation to normal tree growth (Sieghardt *et al.* 2005; Tello *et al.* 2005).

Non-structural carbohydrates (NSC) are believed to improve tolerance to diverse

stress and disturbance conditions (Canham *et al.* 1999; Gleason & Ares 2004; Myers & Kitajima 2007; Atkinson *et al.* 2014; O'Brien *et al.* 2014). During periods of stress for plant growth, NSC can maintain basic metabolic functions, and after disturbances that involve a loss of tissue, NSC can be mobilized from various sources (i.e., stems, leaves, and roots) to potential sinks to maintain metabolism and/or start compensatory growth (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013). NSC concentrations may comprise 5-40 % of the dry matter of a plant, depending on plant functional types and environmental conditions, such as climate and disturbance (Hoch, Richter & Körner 2003; Würth *et al.* 2005; Zhang, Wang & Wang 2014). In general, carbohydrate reserves are comprised of NSC that are formed by low weight sugars and starch. Sugars are mobilized easily and they are used for short-term metabolism, but starch is stored in a more recalcitrant form for long-term use during periods of severe stress (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013).

At the plant level, NSC stores may accumulate passively when carbon supplied by photosynthesis exceeds the carbon demand of the plants (Körner 2003), or plants may accumulate carbon actively when trees regulate the levels of reserves at the expense of growth (Chapin, Schulze & Mooney 1990). Active storage suggests a trade-off between NSC and growth that may influence the carbon allocation "decisions", which may depend on the life-history strategies of trees (Myers & Kitajima 2007; Poorter & Kitajima 2007; Wiley 2013). Therefore, NSC concentrations may be related to other trade-offs linked to the way that plants acquire and invest resources in a manner that has been described as a 'fast-slow' continuum in the plant economics. For example, fast-growing, resource-acquisitive species, are generally characterized by high specific leaf area (SLA), high leaf nutrient concentrations, and low wood density. On the contrary, slow-growing, resource-conservative species, are generally characterized by low SLA, low leaf nutrient concentrations, and high wood density (Grime *et al.* 1997; Westoby *et al.* 2002; Diaz *et al.* 2004; Wright *et al.* 2004; Chave

et al. 2009; Reich 2014; Díaz *et al.* 2016).

At the tissue level, NSC depends on the distance between the carbon sources (NSC pools and currently produced photosynthate) and carbon sinks (respiratory metabolism, storage of NSC, and tissue growth), and the ability of organs to acquire plant-available resources (sink strength) (Lacointe 2000; Minchin & Lacointe 2005). Thus, disturbances that imply loss of tissue will affect the priorities for carbon allocation for growth and reserves. This response will depend on the functional role of the organs involved, because these organs may function as carbon sources or carbon sinks (Li, Hoch & Körner 2002). For instance, after a sudden reduction in photosynthesizing biomass by defoliation or branch pruning, the remaining leaves may increase their photosynthetic rates and their foliar nitrogen to compensate the supply of carbon to growth and storage (Reich *et al.* 1993; Vanderklein & Reich 1999; Quentin *et al.* 2010; Quentin *et al.* 2011). Root pruning reduces the water supply for gas exchange which will inhibit photosynthesis (carbon sources) (Vysotskaya *et al.* 2004) and also produces a reduction in total stored carbohydrates (Landhäusser & Lieffers 2003). Thus, root pruning should cause a reduction in total tree growth, and a reallocation of resources belowground to quickly rebuild its root (Ferree, Scurlock & Schmid 1999; Wajja-Musukwe *et al.* 2008; Dong *et al.* 2016). Stem damage by bark removal affects the mobilization and refilling of reserves between sources and sinks (Högberg *et al.* 2001; Moore 2013; Purcell 2014; Mei *et al.* 2015). Such damage negatively affects the transport of reserves from roots to above-ground parts above the region ring-barked as well as the transport of photosynthates from the foliage to the root system (Moore 2013; Mei *et al.* 2015).

The response of carbohydrate concentrations to simultaneous stresses is more complex since the effect may be more or less severe than the sum of their individual effects (Mittler 2006; Niinemets 2010). Thus, the effect on carbohydrate

concentrations of defoliation and root pruning simultaneously may be lower compared to single stresses. This is because the removal of transpiring leaf area by defoliation reduces the impact of water stress that is caused by root pruning and, thus, reduces the need to use reserves to initiate compensatory growth to produce new roots and to exploit new available soil nutrients and water resources (Quentin *et al.* 2012; Jacquet *et al.* 2014). On the other hand, the combination of tissue loss (by either defoliation or root pruning) and stem damage could lead to a reduction of reserve concentrations, because stem damage would limit the supply of reserves from roots to leaves that is required to initiate compensatory leaf production under defoliation, or it would limit the supply of new photosynthates from leaves to roots required to increase root production (Mei *et al.* 2015).

The purpose of my dissertation was to improve our understanding of the relationship between stress and disturbance, which are faced by trees in urban and natural areas, and NSC storage at the tissue and plant level. The main hypotheses that I wanted to test were (*i*) if there was a coordinated variation between NSC concentrations and the leaf and wood economic spectra, independent of the geographical origin of species (tropical/temperate). Therefore, I expected that species with higher NSC concentrations have trait values that are associated with resource conservation or 'slow' ecological strategies, such as a low SLA, high tissue density, and low concentrations of leaf nutrients, and the reverse for species with trait values that are associated with resource acquisition or 'fast' ecological strategies; (*ii*) that there are negative and positive interactions in NSC concentrations in trees exposed simultaneously to common urban stresses. Thus, compared to single stresses, the effect of combined stress factors should be a decrease in NSC concentrations (negative interaction) as a response to tissue loss (either by defoliation or root pruning) and stem damage, and higher NSC concentrations (positive interaction) in NSC in response to defoliation and root pruning simultaneously.

Initially in ***Chapter 1***, thanks to the large number of samples collected for analysis and the costs and time commitment required for the procedures to measure NSC, I proposed a novel technique for estimating NSC through near-infrared spectrometry. In ***Chapter 2***, I sampled 80 tree species from temperate deciduous, and upper montane and lowland tropical forests to evaluate the large-scale ecological patterns of variation between NSC concentrations and leaf and woody functional traits. In ***Chapter 3***, I measured the seasonal dynamics of NSC in woody tissues of both pruned and un-pruned trees of *Acer saccharinum* and *Acer platanoides* to evaluate the dynamics of NSC concentrations after pruning during a single growing season. Finally, in ***Chapter 4***, I conducted a large scale manipulative experiment with three tree species that are commonly planted in cities of eastern North America (*Fraxinus pennsylvanica*, *Celtis occidentalis* and *Tilia cordata*). These trees suffered different levels of defoliation, root pruning, and stem damage (and their interactions) to evaluate treatment effects on NSC concentrations.

The four chapters of this thesis are presented in the format of scientific journal articles. The first chapter is already published in the journal *Methods in Ecology and Evolution* (Ramirez *et al.* 2015). The second and third chapter will be submitted shortly to *New Phytologist* and *Urban Forestry & Urban Greening*, respectively. The journal to which I will submit the fourth chapter has not been identified yet

1 CHAPTER I

NEAR-INFRARED SPECTROSCOPY (NIRS) PREDICTS NON-STRUCTURAL CARBOHYDRATE CONCENTRATIONS IN DIFFERENT TISSUE TYPES OF A BROAD RANGE OF TREE SPECIES

Jorge A. Ramirez[1], Juan M. Posada[2], I. Tanya Handa[1], Günter Hoch[3], Michael Vohland[4], Christian Messier[1,5] and Björn Reu[6,7]

[1]Center for Forest Research, Université du Québec à Montréal, P.O. Box 8888, Succursale Centre-ville, Montréal, Québec, H3C 3P8, Canada

[2]Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá, Colombia

[3]Institute of Botany, University of Basel, Basel, Switzerland

[4]Geoinformatics and Remote Sensing, Institute for Geography, University of Leipzig, Germany

[5]Institut des Sciences de la Forêt Tempérée (ISFORT), Université du Québec en Outaouais (UQO), Ripon, Quebec, Canada

[6]Spezielle Botanik und Funktionelle Biodiversität, Universität Leipzig, Leipzig, Deutschland

[7]Escuela de Biología, Universidad Industrial de Santander, Bucaramanga, Colombia

Published in *Methods in Ecology and Evolution* (2015) 6: 1018–1025

Key words: carbohydrate reserves, carbon allocation, CARS-PLSR, multivariate calibration, spectroscopy, starch, sugars

1.1 ABSTRACT

The allocation of non-structural carbohydrates (NSCs) to reserves constitutes an important physiological mechanism associated with tree growth and survival. However, procedures for measuring NSC in plant tissue are expensive and time-consuming. Near-infrared spectroscopy (NIRS) is a high-throughput technology that has the potential to infer the concentration of organic constituents for a large number of samples in a rapid and inexpensive way based on empirical calibrations with chemical analysis.

The main objectives of this study were (i) to develop a general NSC concentration calibration that integrates various forms of variation such as tree species and tissue types and (ii) to identify characteristic spectral regions associated with NSC molecules. In total, 180 samples from different tree organs (root, stem, branch, leaf) belonging to 73 tree species from tropical and temperate biomes were analysed. Statistical relationships between NSC concentration and NIRS spectra were assessed using partial least squares regression (PLSR) and a variable selection procedure (competitive adaptive reweighted sampling, CARS), in order to identify key wavelengths.

Parsimonious and accurate calibration models were obtained for total NSC (r^2 of 0·91, RMSE of 1·34% in external validation), followed by starch (r^2 = 0·85 and RMSE = 1·20%) and sugars (r^2 = 0·82 and RMSE = 1·10%). Key wavelengths coincided among these models and were mainly located in the 1740–1800, 2100–2300 and 2410–2490 nm spectral regions.

This study demonstrates the ability of general calibration model to infer NSC concentrations across species and tissue types in a rapid and cost-effective way. The estimation of NSC in plants using NIRS therefore serves as a tool for functional biodiversity research, in particular for the study of the growth–survival trade-off and its implications in response to changing environmental conditions, including growth limitation and mortality.

1.2 INTRODUCTION

Trees store non-structural carbohydrates (NSCs) as reserves to maintain plant metabolism during and after unfavourable conditions for plant growth. NSC reserves allow trees to survive cold temperatures at high altitudes and latitudes, provide energy for vegetative growth in spring and enable recovery and survival following periods of negative carbon balance indu-ced by drought (McDowell et al. 2008; Mitchell *et al.* 2013; O'Brien *et al.* 2014), altitudinal limitation (Handa, Körner & Hättenschwiler 2005; Hoch & Körner 2012; Fajardo & Piper 2014) and disturbances by herbivores (Kobe 1997; Canham *et al.* 1999; Myers & Kitajima 2007; Atkinson *et al.* 2014).

There is considerable knowledge about the dynamics and mobilization of NSC reserves (Hoch, Richter & Körner 2003; Sala, Woodruff & Meinzer 2012; *Dietze et al.* 2013). However, there is little knowledge about the amount of carbon allocated to reserves, whether it is controlled passively or actively, or in which part of the plant reserves are stored (Sala, Woodruff & Meinzer 2012; Dietze *et al.* 2013). Furthermore, variation of NSC allocation to reserves across species and biomes, as well as how it is controlled by the environment or along gradients of seasonality, is also poorly documented (Dietze *et al.* 2013). The dearth of research on the mechanisms driving variation in NSC allocation is likely attributable to the time, labour and costs associated with the methods to measure NSC concentrations in plant tissues (Chow & Landhäusser 2004; Bellasio, Fini & Ferrini 2014). While there are different well-standardized analytical methods for analysing carbohydrates in plant tissues, such as photometry, colorimetric or chromatography (Gomez *et al.* 2003), these methods are expensive and time-consuming (Batten *et al.* 1993; Machado Dugante *et al.* 2013). Additionally, analytic results from these different methods are

not comparable since they depend on the protocols selected to separate carbohydrates from the tissue matrix and the hydrolysis to break down sugars and starch to glucose (Chow & Landhäusser 2004; Bellasio, Fini & Ferrini 2014). This limits the investigation of NSC reserves in a biodiversity context considering a large quantity of samples for many species.

Near-infrared spectroscopy (NIRS) is a high-throughput technique that allows for analysing a large quantity of samples. NIRS can be used to extrapolate measured contents of organic constituents from a limited number to a large number of samples (Foley *et al.* 1998). It measures the absorbance of light at specific wavelengths by different molecular bonds, principally -CH, -OH and –NH, which are the primary constituents of organic compounds of plant tissues (Bokobza 2002). A statistical relationship ('calibration') between the near-infrared spectra and a sub-data set that has been analysed for the components of interest (Foley *et al.* 1998) allows to extrapolate the constituent of interest for a large number of samples expediently (Lawler *et al.* 2006). Hence, NIRS could be ideally suited for analysing NSC concentration in plant tissues, as sample preparation is straightforward and rapid and no chemical reagents are necessary. Furthermore, NIRS measurements also can be used to estimate other parameters of interest, including nitrogen, cellulose and lignin, if biochemical analyses are also available to calibrate a relationship (Gillon, Houssard & Joffre 1999; Petisco *et al.* 2005, 2006). Starch and soluble sugar concentrations have been also determined in shoot samples from rice and wheat as well as in Rumex obtusifolius roots (Decruyenaere *et al.* 2012). However, none of these studies analysed NSC concentration in different tissue types for many woody species simultaneously subjected to varying environmental conditions. In other words, variation of NSC concentration in trees species spanning different life histories and environmental constraints has not yet been integrated in a study in which carbohydrate reserves were analysed with NIRS.

Hence, the main objectives of this study were *(i)* to explore whether general (global) calibration models can be obtained incorporating variation in NSC concentrations across tree species and tissue types and *(ii)* to identify key wavelength and characteristic spectral regions related to NSC molecules using competitive adaptive reweighted sampling (CARS) variable selection in partial least squares regression (PLSR). The latter would allow for a physicochemical interpretation of the obtained models and their potential robustness.

1.3 MATERIALS AND METHODS

1.3.1 Sampling

In 2012, we sampled 82 native tree species from the Mont St-Hilaire Gault Nature Reserve in the province of Quebec, Canada, and in various forest types in the departments of Cundinamarca and Antioquia, Colombia (Table 1.1). Sites were chosen in order to get a contrast in latitude and seasonality (Canada versus Colombia) and altitude (within Colombia). Botanical samples of tropical species were taken for species verification and deposited at the Medellin Botanical Garden. At Mont St. Hilaire, this was not necessary since all trees had been identified and tagged previously.

Table 1.1 Main characteristics of the study sites

Site	Coordinates	Biome	Elevation (m)	Mean annual precipitation (mm)	Mean annual temperature (°C)	Number of species/samples selected for analysis
Laguna Seca, Páramo de Chingaza, Cundinamarca, Colombia	4°41′12″N 73°46′21″W	Paramo (alpine ecosystem)	3500–3700	1950	8	5/9
Hacienda Sabaneta Nature Reserve, Cundinamarca, Colombia	4°32′30″N 74°15′18″W	Upper montane forest	2650–2900	1900	12	26/57
Rio Claro, Antioquia, Colombia	5°54′04″N 74°51′24″W	Lowland tropical rainforest	250–750	4000	26	24/47
Gault Nature Reserve, Mont-Saint-Hilaire, Quebec, Canada	45°32′31″N 73°09′11″W	Deciduous temperate forest	150–300	825 (rain) + 1710 (snow)	6 (16)*	18/67
					Total	73/180

* Mean growing season temperature.

Leaves and wood samples (including branch, stem and root) were collected from three to five mature individuals per species with a diameter at breast height >10 cm for a total of 1271 samples. Young, fully expanded leaves from plants without visible pathogen or herbivore damage were sampled in the morning at the top of trees using a tree trimmer, while wood tissues were sampled during the day. Stem samples were taken with a 4·3-mm-diameter increment borer while branch samples with diameters 2–3 cm were collected using a tree trimmer. Root samples, each approximately 5 cm long, were taken with a 4·3-mm increment borer from large surface roots near the base of the stem. Samples in Canada were taken after bud break (May) and at the end of the growing season (November). In order to capture seasonal trends in NSC reserves in Colombia, samples were taken during the transition between the dry and the rainy seasons (between January and April). Tissues were brought to the laboratory within 8 h after sampling in a cooler with ice packs to reduce tissue respiration. Then, leaf and wood samples were microwaved and oven-dried at 65°C to a constant weight. Finally, samples were ground using a ball mill and a grinder with a 0·5–mm-aperture mesh sieve.

1.3.2 Spectral measurements

All reflectance spectra were measured using a FT-NIR analyzer (Bruker MPA Multi Purpose FT-NIR Analyzer). Spectra were taken from 1300 to 2650 nm to cover a wide spectral range. This includes the spectral region (2000–2650 nm) with high overlapping absorbance peaks, as well as the first, second and third harmonic regions (1300–2000 nm) which are informative regions with lower noise levels (Workman & Weyer 2012). The spectral data were recorded at a mean spectral resolution of 1·7 nm and averaged over 5 scans per sample as absorbance (log 1/R, where R = reflectance).

1.3.3 Biochemical analysis

A subset of 180 samples (out of the 1271 samples) was selected for NSC analysis using the Kennard–Stone algorithm (Kennard & Stone 1969). This algorithm selects a defined number of representative samples that systematically cover the spectral variation of all samples (Table 1.1). Samples from the selected subset were analysed for NSC concentration following Hoch, Popp & Körner (2002). 10 mg of ground plant material was extracted with 2 ml distilled water over steam for 30 min. The sum of the three quantitatively most important low molecular weight sugars (i.e. glucose, fructose and sucrose) was determined in an aliquot of the extract after conversion of sucrose and fructose to glucose with invertase and phosphoglucose-isomerase (both Sigma-Aldrich, St. Louis, MO, USA). Total glucose was quantified in a microplate photometer at 340 nm (Thermo Fisher Scientific, Waltham, MA, USA) after the conversion of glucose to gluconate-6-phosphate using the glucose hexokinase (GHK) assay reagent (G3292, Sigma-Aldrich). The rest of the extract including the pellet was treated with a crude fungal amylase ('Clarase' from *Aspergillus oryzae*; Enzyme Solutions Pty Ltd., Crydon South, VIC, Australia) and incubated at 40°C for 15 h to break down starch to glucose. The sum of free sugars (glucose, fructose and sucrose) and starch (referred here as NSC) was then determined photometrically as described above. Starch was calculated indirectly by subtracting the measured sugars (sugars = glucose + fructose +sucrose) from the measured total NSC. Pure starch and glucose, fructose and sucrose solutions were used as standards. Plant powder from orchard leaves (Leco, St. Joseph, MI, USA) was included to control replicability of the extractions. The NSC concentrations are reported here as the percentage of dry matter.

1.3.4 Statistical analysis

Partial least squares regression (PLSR) was used to develop calibrations for the prediction of sugar, starch and total NSC concentrations in one model for all studied plant tissues and to assess total NSC concentrations for all tissue types separately. We used spectral information between 1300 and 2650 nm, because light absorption of NSC-related molecular bonds usually occurs within this spectral region (Curran 1989). For PLSR analysis, we used the first derivative of the spectra, which led to better results compared to raw or vector-normalized spectra.

For the calibration, a subset of 66% of the samples (n = 120 in all tissue models) was selected using the Kennard–Stone algorithm to mimic our initial selection procedure. The remaining 33% of samples (n = 60 in all tissue models) were set aside for independent (external) validation. Calibration equations were derived using PLSR and a variable selection procedure to find the smallest subset of spectral variables. Variable selection was performed using CARS-PLSR (CARS competitive adaptive reweighted sampling). CARS selects an optimal number of spectral variables, which returns the lowest root-mean-squared error (RMSE), and an optimum number of latent variables using leave-one-out cross-validation (Li *et al.* 2009). As CARS uses a Monte Carlo subsampling strategy and random numbers in the adaptive reweighted sampling procedure, no unique solution exists. Therefore, we used 50 CARS simulations to identify the best model, that is the model with the lowest RMSE in cross-validation. We used a maximum of 12 latent variables and selected the best CARS-PLSR model, which was subsequently applied to the external validation set. Model performance was assessed by using the coefficient of determination (Pearson's r^2) and the root-mean-squared error (RMSE). Additionally, the residual prediction deviation (RPD, the ratio of standard deviation of the prediction values to standard error) in PLSR calibration, cross-validation and the external validation was

calculated. RPD is a quality measure of the model performance for predicting carbohydrate concentrations. In contrast to RMSE which is given in the unit of the response variable, RDP is independent from it and is thus more general measure allowing comparisons between calibration models considering different response variables and ranges within their concentrations (Saeys, Mouazen & Ramon 2005). Predictions from models with RPD values between 1·5 and 2·0 allow one to differentiate between high and low values, while RPD values higher than 2·5 yield good to excellent predictions. All statistical analyses were performed in R 3.1.1 (R Development Core Team, Vienna, Austria, 2014) using the packages *pls*, *soil.spec* and *carspls*.

1.4 RESULTS

1.4.1 Predicting NSC across leaves, stems and roots for all species (all tissue models)

The concentration of carbohydrates expressed in % dry matter in the sub-data set ranged between 0 and 13·5% in sugars (the sum of glucose, fructose and sucrose), 0 and 17·8% in starch, and 0·1 and 20·1% in total NSC (sugars plus starch). For all three-carbohydrate constituents, robust and parsimonious calibration models were identified using CARS-PLSR that retained their predictive power in the external validation (Figure 1.1 and 1.2). Across all calibration models, the best models were obtained for total NSC (r^2 of 0·91, RMSE of 1·34% in validation), followed by starch (r^2 = 0·85 and RMSE = 1·20%) and sugars (r^2 = 0·82 and RMSE = 1·10%) (Table 1.2). The number of latent variables varied between 10 and 12 and the number of selected predictor variables between 24 and 42, corresponding to 3·7–8·3% of all spectral bands, respectively. The best models consistently indicated parsimonious and accurate calibration with RPD values greater than 2 (RPD values higher than 2·0 indicate good to excellent predictions, Saeys, Mouazen & Ramon 2005).

Table 1.2 Description of CARS-PLSR regression models per carbohydrate type.

Carbohydrate - tissue type	Free sugars - all tissues (%)	Starch - all tissues (%)	Total NSC - all tissues (%)	Total NSC - leaves (%)	Total NSC – stem and branches (%)	Total NSC - roots (%)
Latent variables	12	11	11	10	12	10
Predictor variables (#)	24	37	42	33	24	19
r^2 calibration	0.81	0.9	0.93	0.98	0.97	0.99
r^2 cross-validation	0.69	0.84	0.88	0.94	0.93	0.94
r^2 validation	0.82	0.85	0.91	0.68	0.87	0.91
RMSE calibration	0.89	1.18	1.12	0.71	0.67	0.55
RMSE cross-validation	1.14	1.5	1.43	1.12	1	1.11
RMSE validation	1.1	1.2	1.34	2.63	1.22	1.18
RPD cross-validation	1.8	2.5	2.88	4.22	3.86	4.22
RPD validation	2.32	2.53	3.26	1.45	2.58	3.41
Bias* validation	-0.27	-0.23	0.43	-0.93	0.1	0.3

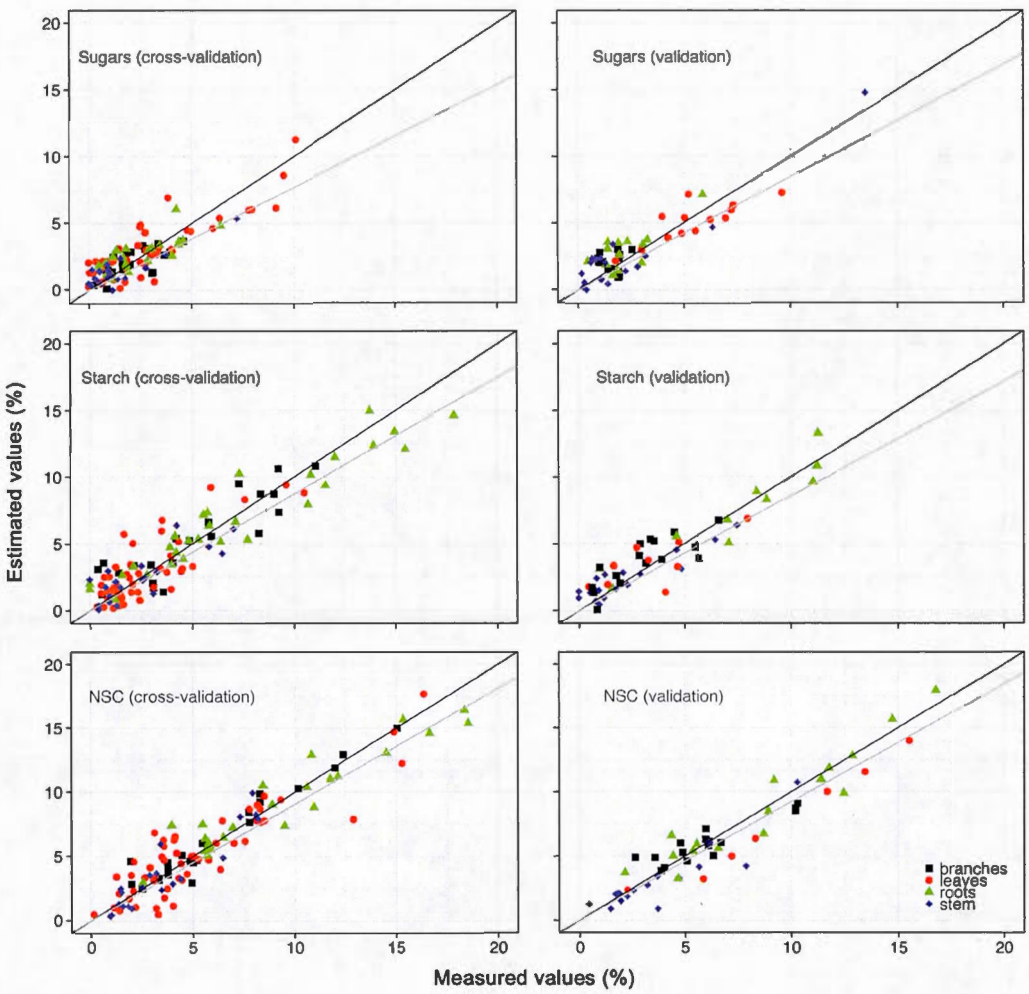

Figure 1.1 CARS-PLSR regression models for sugars, starch and total non-structural carbohydrate concentrations in plant tissues (root, stem, branch, leaf).

Sample sizes for cross-validation (left) and independent validation models (right) were n = 120 and 60, respectively. Linear fits in grey and 1:1 lines in black.

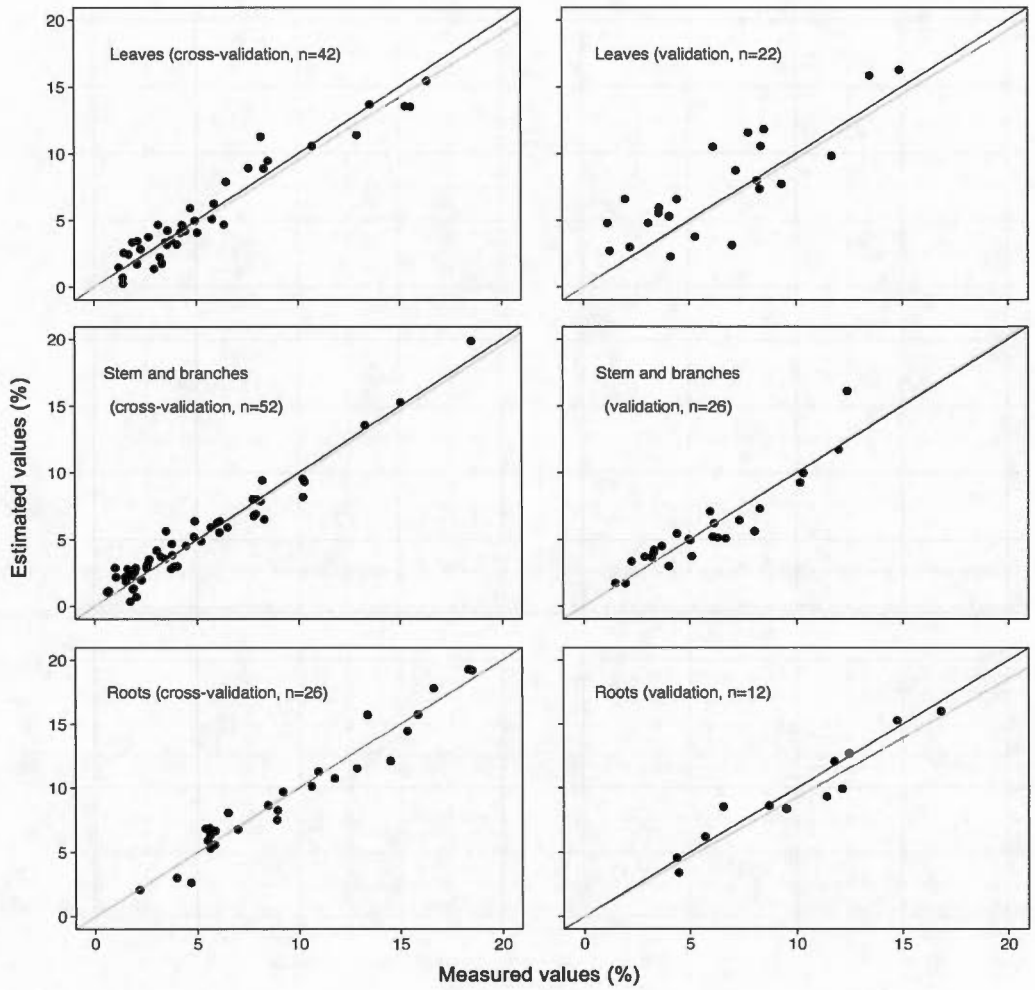

Figure 1.2 CARS-PLSR regression models for total non-structural carbohydrate concentration for tissue-specific models. Cross-validation and validation are shown left and right, respectively. Linear fits in grey and 1:1 lines in black.

The CARS-PLSR algorithm selected a set of wavelengths that explained the greatest amount of variation. To predict sugars, starch and NSC across all tissue types, 24, 37 and 42 wavelength bands were selected, respectively. Wavelengths with high regression coefficients (see bar plots indicating the relative weight of regression coefficients of each selected wavelength, Fig. 3) coincided among sugar, starch and total NSC models and are mainly located in the 1740–1800, 2100–2300 and 2410–2490 nm spectral regions.

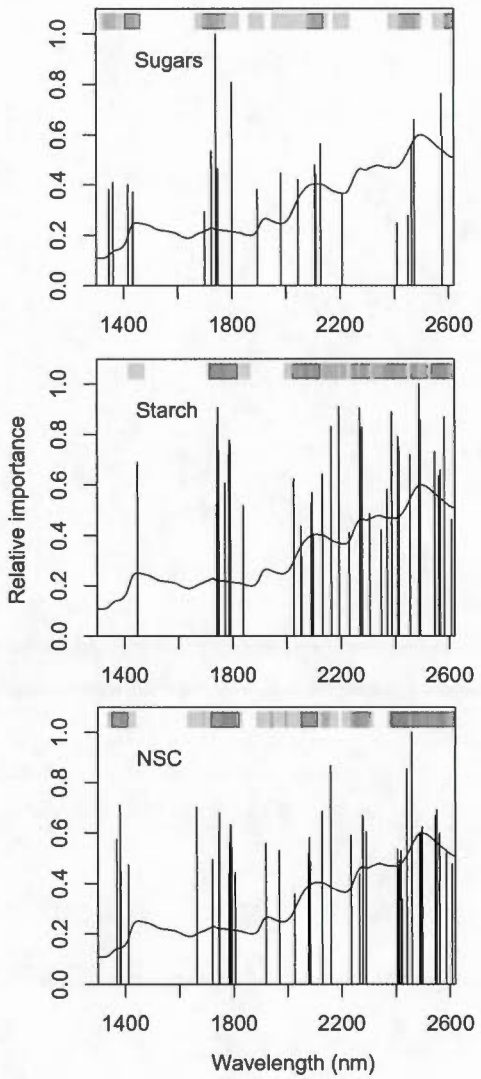

Figure 1.3 Positions of the key wavelengths in the NIR spectrum. Bars indicate the relative importance of the regression coefficients of each key wavelength. Superimposed are the mean calibration spectra.

1.4.2 Predicting NSC for leaves, stems or roots for all species (tissue-specific models)

The tissue-specific models (considering the three different tissue types separately with respect to total NSC concentration) differed in terms of accuracy and parsimony (Table 1.2). The best model was obtained for total NSC in roots (r^2 = 0·91 and RMSE = 1·18%), followed by stem and branches, and leaves. NSC concentration of leaves showed the poorest accuracy among all models with an RPD of 1·45. For example, the NSC model for leaves had the lowest number of latent variables (10) and relatively few predictor variables (33); in the external validation, its accuracy was the poorest. In contrast, the NSC model for roots with 10 latent variables and 19 predictor variables had the highest accuracy of all considered models. The number of predictor variables varied between 19 and 42, and the highest bias was found in the NSC model for leaves, while the lowest was found for the NSC model of stem and branches (Table 1.2).

1.5 DISCUSSION

Carbohydrate concentrations of the samples in this study were comparable to the range recorded in other studies across the globe (0–19 NSC (% d.m.), Hoch & Körner 2003; Hoch, Richter & Körner 2003, 2003; Landhäusser & Lieffers 2003; Würth *et al.* 2005; Piper *et al.* 2009). Despite substantial variation in NSC concentrations across species, tissue types and environmental conditions (Table 1) we were able to identify accurate and robust calibration models for total NSC, sugar and starch (Table 1.2). The CARS-PLSR variable selection efficiently identified a relatively small set of predictor variables (ranging between 19 and 42) and the identification of key wavelengths. We consider this step, together with the representative sample selection using the Kennard–Stone algorithm, as crucial for yielding parsimonious and robust calibrations.

The performance of our calibration models was equivalent to or better than those reported in previous studies that estimated carbohydrate concentration in plant tissues using NIRS (Table 1.2). For example, Batten *et al.* (1993) reported r^2 = 0·98 and RMSE = 1·4% in calibration for NSC estimation in rice and wheat. Decruyenaere *et al.* (2012) reported r^2 values between 0·96 and 0·98 and RMSE between 0·63 and 1·85% in calibration and cross-validation models for estimating sugar and starch in Rumex obtusifolius roots. However, their relationships did not perform well in independent validation, as indicated by a low r2 and high standard error of prediction (r^2 = 0·003 and SE of prediction = 2·7). Conversely, Chen *et al.* (2014) reported r^2 = 0·81 and RMSE = 1·77% in external validation of models for estimating sucrose and glucose concentration in sorghum stalks. Relative to other studies, the robustness of our models in the independent validation (as compared to the external validation)

provides strong support for using our calibration models to estimate NSC concentration of plant species across broad environmental gradients and life histories.

Unlike other studies using NIRS to estimate carbohydrates or other parameters such as nitrogen, cellulose and lignin (Gillon, Houssard & Joffre 1999; Petisco *et al.* 2005, 2006; Klaus *et al.* 2012), we did not use commercial software for data processing. Commercial software often performs automated variable selection or pre-processing (sometimes called optimization) and does not allow users to adjust these procedures. The automation of these procedures may lead to over-fitting or spurious results when results are not validated externally. All analyses performed in this study were done in R with well-documented procedures that allow for replication and verification of the results obtained here.

The performance of NIRS calibrations is usually evaluated using the root-mean-squared error (RMSE) in either cross-validation or external validation. In this study, the best calibration was found for tissue-specific NSC models (Table 1.2 and Figure 1.2). However, RMSE did not vary much across the different models (except the model for leaves) when using external validation. Another option to evaluate model performance is RPD. RPD values obtained in external validation in this study were higher than 2·0 indicating a very good model performance, except for total NSC in leaves, where RPD was 1·45. As RPD values for starch (all tissues) and NSC (stem and branches, roots and all tissues) models were greater than 2·5, the performance of these models can be considered very reliable and useful for ecological research within and across ecosystems and biomes (Saeys, Mouazen & Ramon 2005). The small differences in RMSE and RPD values between cross-validation and external validation provide evidence that the Kennard–Stone algorithm is a useful algorithm to select representative calibration samples. Thus, we recommend using this algorithm

to select samples prior to NIRS analysis in cases where not all samples can be analysed and extrapolation is wanted.

Sample heterogeneity is vital for optimal calibration, as it ensures that NIRS can be used to estimate NSC concentration across a broad range of plant species and tissue types. Contrary to the concern that samples from different biomes or tissues may lead to bias in validation and cross-validation results (Cécillon *et al.* 2009), our calibrated models for this wet chemistry method were accurate and robust for most tissue types. The exception to this was the relatively poor performance of the leaves model (validation RPD = 1·45). This may be due to the presence of phenolics or tannins that in some species can interfere with enzymatic techniques disrupting the signal intensity and leading to biased measurements in chemical analysis (Ashwell 1957). Furthermore, the presence of primary metabolites that were not measured with our NSC method may obscure the NSC measurements. For example, this may be caused by neutral lipids (Hoch, Richter & Körner 2003) and other secondary metabolites in living leaves that are responsible for defence against herbivores, protection against ultraviolet radiation or high temperatures (Lambers, Chapin & Pons 2008). In addition, secondary metabolites may be detected at spectral regions normally associated with carbohydrates and, thus, may also obscure the NIRS–carbohydrate relationships (Curran 1989; Workman & Weyer 2012). This finding suggests that further research is needed on chemical compounds inferring with NSC content in either wet chemistry analysis or spectral measurements.

The reflectance spectrum of a sample is the result of the absorption features of each chemical compound, weighted by its concentration. Identified reflectance of key wavelengths corresponds to the regions within the light spectrum of high correlation between reflectance and chemical concentration (Curran 1989). These wavelengths constitute the spectral frequencies that produce the minimum errors during

quantitative determinations and may help to discriminate between dissimilar samples (Xiaobo *et al.* 2010; Workman & Weyer 2012). Key wavelengths identified in this work were located in the long-wavelength NIR region, precisely between 1740–1800, 2100–2300 and 2410–2490 nm.

In the case of biological samples, overlapping absorptions are typically generated by overtones and combinations of vibrations of organic matter functional groups like C-H, O-H and N-H (Siesler *et al.* 2002; Chung, Boik & Potma 2013). The regions identified in this study were mainly related to the stretching and bending vibrations of the molecular bonds between hydrogen atoms and oxygen (O-H group regions) (Curran 1989; Siesler *et al.* 2002; Workman & Weyer 2012). In this case, the organic compounds that absorb in these wavelengths and O-H bond vibrations are related to the chemical concentrations of cellulose, sugar and starch, and lignin (Curran 1989; Batten *et al.* 1993; Decruyenaere *et al.* 2012; Workman & Weyer 2012).

Physiological research about the role of NSC in plants has mainly focused on free sugars (such as glucose, fructose and sucrose) and starch. In general, free sugars are used by plants for cellular metabolism, while starch is stored in a more recalcitrant form that must be transformed to a labile form before being transported or metabolized (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013). Little is known about mechanisms that control the transformation between starch and sugar and their different roles in plant functioning (Kobe 1997; Ogle & Pacala 2009; Dietze *et al.* 2013). Despite the importance of this chemical process, many studies have not differentiated NSCs into sugars and starch. However, our method readily detects sugars and starch, which can provide further insight to important physiological processes in plants. This may facilitate the analysis of multiple samples required to

resolve outstanding questions about the role of carbohydrate reserves along the growth–survival trade-off and its variation among functional groups (Poorter & Kitajima 2007; Dietze *et al.* 2013).

1.6 CONCLUSIONS

To date, estimating carbohydrate concentrations for plant tissues using NIRS has been restricted to a handful of studies that have focused on annual plants in a single biome (Batten *et al.* 1993; Decruyenaere *et al.* 2012; Chen *et al.* 2014). This study presents a successful application of a NIRS-based NSC quantification considering many woody species, different tissue types and a broad range of environmental conditions. CARS-PLSR variable selection of key spectral regions yielded consistent, parsimonious and robust calibrations across the three NSC constituents. Our results show that this approach for estimating plant carbohydrates with NIRS and is a promising avenue for physiological and ecological studies covering a wide range of species in different biomes, particularly the study of the growth–survival trade-off and its implications.

1.7 ACKNOWLEDGEMENTS

This research was supported by the NSERC/Hydro-Quebec research chair on tree growth control and by a scholarship from the Quebec research funds for nature and technology. We would like to thank Christian Körner, Christian Wirth and Alexandra Weigelt for their support to facilitate our project. We thank Nathaly Guerrero for helpful discussion and Dylan Craven for critically reading the manuscript.

1.8 SUPPORTING INFORMATION

1.8.1 Annex A

Table 1.3A Species list.

Number	Species	Biome
1	*Acer pensylvanicum*	Temperate forest
2	*Acer rubrum*	Temperate forest
3	*Acer saccharinum*	Temperate forest
4	*Acer saccharum*	Temperate forest
5	*Aegiphila bogotensis*	Montane tropical forest
6	*Ageratina ampla*	Paramo
7	*Alnus acuminata*	Montane tropical forest
8	*Alnus rugosa*	Temperate forest
9	*Aniba perutilis*	Lowland tropical forest
10	*Apeiba glabra*	Lowland tropical forest
11	*Aptandra tubicina*	Lowland tropical forest
12	*Aspidosperma megalocarpon*	Lowland tropical forest
13	*Befaria resinosa*	Montane tropical forest
14	*Bellucia pentamera*	Lowland tropical forest
15	*Betula alleghaniensis*	Temperate forest
16	*Betula papyrifera*	Temperate forest
17	*Brosimum utile*	Lowland tropical forest
18	*Cariniana pyriformis*	Lowland tropical forest
19	*Carya cordiformis*	Temperate forest
20	*Casearia arborea*	Lowland tropical forest
21	*Cavendishia cordifolia*	Montane tropical forest
22	*Cecropia peltata*	Lowland tropical forest
23	*Cedrela montana*	Montane tropical forest
24	*Celtis occidentalis*	Temperate forest
25	*Cespedesia spathulata*	Lowland tropical forest
26	*Chusquea tessellata*	Paramo
27	*Citharexylum spp.*	Montane tropical forest
28	*Clathrotropis brachypetala*	Montane tropical forest
29	*Clusia multiflora*	Montane tropical forest
30	*Cordia alliodora*	Lowland tropical forest
31	*Cordia cylindrostachya*	Montane tropical forest

Number	Species	Biome
32	*Cordia spp.*	Montane tropical forest
33	*Croton killipianus*	Lowland tropical forest
34	*Drimys granadensis*	Montane tropical forest
35	*Duguetia antioquensis*	Lowland tropical forest
36	*Espeletia grandiflora*	Paramo
37	*Fagus grandifolia*	Temperate forest
38	*Fraxinus americana*	Temperate forest
39	*Gaiadendron tagua*	Montane tropical forest
40	*Gaultheria spp.*	Paramo
41	*Goupia glabra*	Lowland tropical forest
42	*Hieronyma alchorneoides*	Lowland tropical forest
43	*Hyptidendron arboreum*	Lowland tropical forest
44	*Ilex nervosa*	Montane tropical forest
45	*Juglans cinerea*	Temperate forest
46	*Juglans neotropica*	Montane tropical forest
47	*Ladembergia spp.*	Lowland tropical forest
48	*Miconia biappendiculata*	Montane tropical forest
49	*Morella parvifolia*	Montane tropical forest
50	*Myrcianthes leucoxyla*	Montane tropical forest
51	*Myrsine coriacea*	Montane tropical forest
52	*Myrsine ferruginea*	Montane tropical forest
53	*Ochoterenaea colombiana*	Lowland tropical forest
54	*Ochroma pyramidale*	Lowland tropical forest
55	*Oreopanax bogotensis*	Montane tropical forest
56	*Ostrya virginiana*	Temperate forest
57	*Piper bogotense*	Montane tropical forest
58	*Populus grandidentata*	Temperate forest
59	*Populus tremuloides*	Temperate forest
60	*Prunus buxifolia*	Montane tropical forest
61	*Prunus serotina*	Temperate forest
62	*Pseudoxandra sclerocarpa*	Lowland tropical forest
63	*Quercus rubra*	Temperate forest
64	*Rhamnus goudotiana*	Montane tropical forest
65	*Senecio spp.*	Paramo
66	*Solanum humboldtianum*	Montane tropical forest
67	*Tabebuia guayacan*	Lowland tropical forest
68	*Tapirira guianensis*	Lowland tropical forest
69	*Tilia americana*	Temperate forest
70	*Trema micrantha*	Lowland tropical forest
71	*Verbesina crassiramea*	Montane tropical forest
72	*Viburnum lasiophyllum*	Montane tropical forest

Number	Species	Biome
73	*Vismia macrophylla*	Lowland tropical forest

1.8.2 Annex B

Table 1.4B CARS-PLSR regression equiations.

General model equation: $y= \beta_1 X_1+ \beta_2 X_2+\cdots+\beta_n X_n$; where:

y : Carbohydrate analyzed
βi: Coefficient value
Xi: Reflectance at wavelength considered
n: Number of variables considered

	Sugars all tissues		**Starch all tissues**		**NSC all tissues**		**NSC in leaves**		**NSC in stem and branches**		**NSC in roots**	
i	Wavelength considered	Coefficient value	Wavelength considered	Coefficient value	Wavelength considered	Coefficient value	Wavelength considered	Coefficient value	Wavelength considered	Coefficient value	Wavelength considered	Coefficient value
1	1346.1	1401.52	1448.4	-1947.04	1368.9	1652.48	1418.3	2539.21	1389.4	2548.64	1692.3	-4938.22
2	1360.2	1505.30	1740	1481.92	1380.5	2048.54	1432.4	2820.20	1679.2	-1799.78	1744.7	3747.59
3	1416.7	-1482.09	1742.4	2365.37	1383.5	1291.04	1474.8	-1247.07	1681.3	-3566.24	1747.1	3772.48
4	1434.0	1136.99	1744.7	2552.34	1412.1	-1360.91	1476.4	-1518.19	1775.8	-2958.57	1749.4	3003.39
5	1435.6	1365.18	1747.1	2080.41	1664.1	2019.27	1484.9	-2082.86	1778.2	-2925.09	1903.5	-2725.21
6	1699.0	-1075.29	1770.9	1710.27	1721.5	-1418.94	1486.6	-1814.65	1808.0	-2384.78	1943.5	-3368.90
7	1723.8	-1965.37	1785.6	-2034.80	1744.7	1739.66	1681.3	2126.46	1810.5	-2144.45	1970.1	4933.21
8	1740.0	-3675.64	1788	-2190.16	1747.1	1955.02	1701.2	-1520.17	1881.4	-2885.63	2009.8	2769.26
9	1747.1	-1709.09	1790.5	-2146.69	1783.1	-1266.13	1703.4	-1052.40	2006.7	-1749.92	2012.9	2940.24

	Sugars all tissues		Starch all tissues		NSC all tissues		NSC in leaves		NSC in stem and branches		NSC in roots	
10	1800.4	-2971.25	1838.7	-1461.89	1785.6	-1622.83	1744.7	2582.41	2047.9	3150.90	2132.1	4939.72
11	1895.2	-1402.59	2025.5	1759.52	1788.0	-1819.92	1768.5	1717.27	2135.6	-2714.44	2298.4	2349.90
12	1982.1	-1641.81	2051.1	-1233.34	1790.5	-1812.15	1770.9	1876.24	2262.3	-2128.47	2382.9	-4569.69
13	2044.7	1552.81	2054.4	-887.62	1793.0	-1561.13	1773.3	1928.10	2278.2	3035.93	2414.0	4197.53
14	2107.8	-1760.64	2090.8	1489.50	1805.4	-1274.98	1775.8	1684.87	2298.4	-4015.98	2418.5	3692.82
15	2111.3	-1633.96	2094.2	1605.88	1920.5	-1610.96	1788.0	-2056.36	2335.7	3354.69	2517.1	-7132.23
16	2128.6	2077.88	2097.6	1146.25	1970.1	1525.36	1802.9	-1473.31	2414.0	3099.00	2561.9	3915.74
17	2208.4	-1345.11	2132.1	1812.48	2025.5	1030.77	1961.1	1833.45	2418.5	3499.83	2587.4	5161.28
18	2409.5	-918.69	2164.1	-2345.91	2077.4	1495.55	1976.1	1896.36	2455.1	-1305.74	2618.8	6104.91
19	2450.5	1019.75	2193.4	2563.72	2080.8	1675.19	1979.1	1955.32	2488.1	-2642.78	2624.1	4062.48
20	2464.5	-2030.95	2231.2	-1160.82	2084.1	1396.00	2104.4	-2158.92	2492.9	-2425.82		
21	2473.9	2425.12	2270.2	-2548.62	2128.6	1976.82	2242.8	-1265.95	2497.7	-1607.15		
22	2577.2	2800.26	2278.2	2335.64	2160.5	-2494.51	2270.2	-1935.49	2526.9	1525.80		
23	2618.8	1458.98	2306.6	1370.77	2235.0	-1699.64	2278.2	1743.74	2531.9	2758.84		
24	2624.1	2041.93	2348.4	-1188.33	2266.3	-1251.80	2323.1	-1438.82	2556.8	-3113.36		
2			2369.9	1643.76	2278.2	1922.19	2327.3	-2664.78				

Sugars all tissues	Starch all tissues		NSC all tissues		NSC in leaves		NSC in stem and branches	NSC in roots
	2387.3	-2506.32	2290.3	-1743.22	2488.1	-2237.89		
	2409.5	2230.96	2405.0	1539.91	2492.9	-2219.72		
	2414	2114.69	2409.5	1315.02	2546.8	-1650.53		
	2455.1	-2026.37	2414.0	1414.84	2556.8	-861.51		
	2488.1	-2821.97	2418.5	1523.92	2577.2	1333.27		
	2492.9	-2425.77	2423.0	972.47	2608.3	-2753.90		
	2546.8	-2060.55	2441.3	-2457.44	2640.1	1835.52		
	2561.9	-1796.34	2459.8	-2877.83	2645.5	1917.02		
	2567	-1857.18	2488.1	-1726.38				
	2582.3	2451.14	2492.9	-1726.81				
	2608.3	-1301.37	2497.7	-1795.05				
	2645.5	1316.55	2546.8	-1922.48				
			2551.8	-1994.77				
			2561.9	-1724.34				
			2587.4	1498.08				

	Sugars all tissues	Starch all tissues	NSC all tissues		NSC in leaves	NSC in stem and branches	NSC in roots
41			2608.3	-1373.91			
42			2645.5	1661.82			

1.9 REFERENCES

Ashwell, G. (1957) Colorimetric analysis of sugars. *Methods in Enzymology 3* (eds N.P. Kaplan & S.P. Colowick), pp. 73-105. Elsevier Academic Press, New York.

Atkinson, R.R.L., Burrell, M.M., Rose, K.E., Osborne, C.P. & Rees, M. (2014) The dynamics of recovery and growth: how defoliation affects stored resources. *Proceedings of the Royal Society B,* **281,** 20133355.

Batten, G.D., Blakeney, A.B., McGrath, V.B. & Ciavarella, S. (1993) Non-structural carbohydrate: Analysis by near infrared reflectance spectroscopy and its importance as an indicator of plant growth. *Plant and Soil,* **155/156,** 243-246.

Bellasio, C., Fini, A. & Ferrini, F. (2014a) Evaluation of a High Throughput Starch Analysis Optimised for Wood. *PLoS ONE,* **9,** e86645.

Bellasio, C., Fini, A. & Ferrini, F. (2014b) Evaluation of a High Throughput Starch Analysis Optimised for Wood. *PLoS ONE,* **9,** e86645.

Bokobza, L. (2002) Origin of Near-Infrared Absorption Bands. *Near-Infrared Spectroscopy: Principles, Instruments, Applications* (eds H.W. Siesler, Y. Ozaki, S. Kawata & H.M. Heise). Wiley-VCH, Weinheim (Germany).

Canham, C.D., Kobe, R.K., Latty, E.F. & Chazdon, R.L. (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. *Oecologia,* **121,** 1-11.

Cécillon, L., Barthès, B.G., Gomez, C., Ertlen, D., Genot, V., Hedde, M., Stevens, A. & Brun, J.J. (2009) Assessment and monitoring of soil quality using near-infrared reflectance spectroscopy (NIRS). *European Journal of Soil Science,* **60,** 770–784.

Chapin, F.S., Schulze, E.D. & Mooney, H.A. (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics,* **21,** 423-447.

Chen, S.F., Danao, M.G.C., Singh, V. & Brown, P.J. (2014) Determining sucrose and glucose levels in dual-purpose sorghum stalks by Fourier transform near infrared (FT-NIR) spectroscopy. *Journal of the Science of Food and Agriculture.*

Chow, P.S. & Landhäusser, S.M. (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology,* **24,** 1129–1136.

Chung, C.-Y., Boik, J. & Potma, E.O. (2013) Biomolecular Imaging with Coherent Nonlinear Vibrational Microscopy. *Annual Review of Physical Chemistry,* **64,** 77–99.

Coleman, S.W., Barton, F.E. & Meyer, R.D. (1982) Calibration of a near-infrared spectrometer for prediction of forage quality. pp. 104–105. Oklahoma State University. Agricultural Experiment Station.

Curran, P.J. (1989) Remote Sensing of Foliar Chemistry. *Remote sensing of environment,* **30,** 271- 278.

Decruyenaere, V., Clément, C., Agneessens, R., Losseau, C. & Stilmant, D. (2012) Development of near-infrared spectroscopy calibrations to quantify starch and soluble sugar content in the roots of Rumex obtusifolius. *Weed Research,* **52,** 1–5.

Dietze, M.C., Sala, A., Carbone, M.S., Czimczik, C.I., Mantooth, J.A., Richardson, A.D. & Vargas, R. (2013) Nonstructural Carbon in Woody Plants. *Annual Review of Plant Biology,* **65,** 2.1–2.21.

Fajardo, A. & Piper, F.I. (2014) An experimental approach to explain the Southern Andes elevational treeline. *American Journal of Botany,* **101,** 788–795.

Foley, W.J., McLlwee, A., Lawler, I., Aragones, L., Woolnough, A.P. & Berding, N. (1998) Ecological applications of near-infrared reflectance spectroscopy — a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. *Oecologia,* **116,** 293–305.

Gillon, D., Houssard, C. & Joffre, R. (1999) Using near-infrared reflectance spectroscopy to predict carbon, nitrogen and phosphorus content in heterogeneous plant material. *Oecologia,* **118,** 173-182.

Gomez, L., Jordan, M.O., Adamowicz, S., Leiser, H. & Pagès, L. (2003) Du prélèvement au dosage: réflexions sur les problèmes posés par la mesure des glucides non structuraux chez les végétaux ligneux. *Cahiers Agricultures,* **12,** 369-386.

Handa, T., Körner, C. & Hättenschwiler, S. (2005) A test of the treeline carbon limitation hypothesis by in situ CO2 enrichment and defoliation. *Ecology,* **86,** 1288-1300.

Hoch, G. & Körner, C. (2003) The carbon charging of pines at the climatic treeline: a global comparison. *Oecologia,* **135,** 10-21.

Hoch, G. & Körner, C. (2012) Global patterns of mobile carbon stores in trees at the high-elevation tree line. *Global Ecology and Biogeography,* **21,** 861-871.

Hoch, G., Popp, M. & Körner, C. (2002) Altitudinal increase of mobile carbon pools in Pinus cembra suggests sink limitation of growth at the Swiss treeline. *Oikos,* **98,** 361–374.

Hoch, G., Richter, A. & Körner, C. (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell and Environment,* **26,** 1067-1081.

Kennard, R.W. & Stone, L.A. (1969) Computer Aided Design of Experiments. *Technometrics,* **11,** 137-148.

Klaus, V.H., Kleinebecker, T., Boch, S., Müller, J., Socher, S.A., Prati, D., Fischer, M. & Hölzel, N. (2012) NIRS meets Ellenberg's indicator values: Prediction of moisture and nitrogen values of agricultural grassland vegetation by means of near-infrared spectral characteristics. *Ecological Indicators,* **14,** 82–86.

Kobe, R.K. (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos,* **80,** 226-233.

Körner, C. (2003) Carbon limitation in trees. *Journal of Ecology,* **91,** 4-17.
Lambers, H., Chapin, F.S. & Pons, T.L. (2008) *Plant Physiological Ecology.* Springer, New York.
Landhäusser, S.M. & Lieffers, V.J. (2003) Seasonal changes in carbohydrate reserves in mature northern Populus tremuloides clones. *Trees,* **17,** 471–476.
Lawler, I.R., Aragones, L., Berding, N., Marsh, H. & Foley, W.J. (2006) Near-infrared reflectance spectroscopy is a rapid, cost-effective predictor of seagrass nutrients. *Journal of Chemical Ecology,* **32,** 1353–1365.
Li, H., Liang, Y., Xu, Q. & Cao, D. (2009) Key wavelengths screening using competitive adaptive reweighted sampling method for multivariate calibration. *Analytica Chimica Acta,* **648,** 77–84.
Machado Dugante, F., Higuchi, N., Almeida, A. & Vicentini, A. (2013) Species Spectral Signature: Discriminating closely related plant species in the Amazon with Near-Infrared Leaf-Spectroscopy. *Forest Ecology and Management,* **291,** 240–248.
McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yepez, E.A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist,* **178,** 719-739.
Mitchell, P.J., O'Grady, A.P., Tissue, D.T., White, D.A., Ottenschlaeger, M.L. & Pinkard, E.A. (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytologist,* **197,** 862-872.
Myers, J.A. & Kitajima, K. (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology,* **95,** 383-395.
O'Brien, M.J., Leuzinger, S., Philipson, C.D., Tay, J. & Hector, A. (2014) Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate Change,* **4,** 710-714.
Ogle, K. & Pacala, S.W. (2009) A modeling framework for inferring tree growth and allocation from physiological, morphological and allometric traits. *Tree Physiology,* **29,** 587–605.
Petisco, C., García-Criado, B., Mediavilla, S., Vázquez de Aldana, B.R., Zabalgogeazcoa, I. & García-Ciudad, A. (2006) Near-infrared reflectance spectroscopy as a fast and non-destructive tool to predict foliar organic constituents of several woody species. *Analytical and Bioanalytical Chemistry,* **386,** 1823–1833.
Petisco, C., Garcia-Criado, B., Vázquez de Aldana, B.R., Zabalgogeazcoa, I., Mediavilla, S. & García-Ciudad, A. (2005) Use of near-infrared reflectance spectroscopy in predicting nitrogen, phosphorus and calcium contents in

heterogeneous woody plant species. *Analytical and Bioanalytical Chemistry,* **382,** 458–465.

Piper, F.I., Reyes-Díaz, M., Corcuera, L.J. & Lusk, C.H. (2009) Carbohydrate storage, survival, and growth of two evergreen Nothofagus species in two contrasting light environments. *Ecological Research,* **24,** 1233-1241.

Poorter, L. & Kitajima, K. (2007) Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology,* **88,** 1000-1011.

Saeys, W., Mouazen, A.M. & Ramon, H. (2005) Potential for onsite and online analysis of pig manure using visible and near infrared spectroscopy. *Biosystems Engineering,* **91,** 393–402.

Sala, A., Woodruff, D.R. & Meinzer, F. (2012) Carbon dynamics in trees: feast or famine? *Tree Physiology,* **32,** 764–775.

Shenk, J.S., Workman, J.J. & Westerhaus, M.O. (2008) Application of NIR spectroscopy to agricultural products. *Handbook of near-infrared analysis* (eds D.A. Burns & E.W. Ciurczak). CRC Press, Boca Raton.

Siesler, H.W., Ozaki, Y., Kawata, S. & Heise, H.M. (2002) *Near-infrared spectroscopy: Principles, instruments, applications.* WILEY-VCH, Weinheim.

Workman, J. & Weyer, L. (2012) *Practical guide and spectral atlas for interpretive near-infrared spectroscopy.* CRC Press, Boca Raton.

Würth, M.K.R., Pelaez-Riedl, S., Wright, S.J. & Körner, C. (2005) Non-structural carbohydrate pools in a tropical forest. *Oecologia,* **143,** 11–24.

Xiaobo, Z., Jiewena, Z., Poveyb, M.J.W., Holmes, M. & Hanpin, M. (2010) Variables selection methods in near-infrared spectroscopy. *Analytica Chimica Acta,* **667,** 14–32.

2 CHAPTER II

NON-STRUCTURAL CARBOHYDRATES IN WOODY TISSUES ARE NOT COORDINATED WITH FUNCTIONAL TRAITS IN TEMPERATE AND TROPICAL ANGIOSPERMS

Jorge A. Ramirez[1], Juan M. Posada[2], Tanya Handa [1], Dylan Craven[3], Björn Reu[4,5], Carlos Sierra[6] Günter Hoch [7] and Christian Messier [1,8]

[1]Center for Forest Research, Université du Québec à Montréal, P.O. Box 8888, Succursale Centre-ville, Montréal, Québec, H3C 3P8, Canada

[2]Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá, Colombia

[3]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Platz 5e, 04103 Leipzig, Germany

[4]Spezielle Botanik und Funktionelle Biodiversität, Universität Leipzig, Leipzig, Deutschland

[5]Escuela de Biología, Universidad Industrial de Santander, Bucaramanga, Colombia

[6]Max Planck Institute for Biogeochemistry, Hans-Knöll-Str. 10, 07745 Jena, Germany

[7]Institute of Botany, University of Basel, Basel, Switzerland

[8]Institut des Sciences de la Forêt Tempérée (ISFORT), Université du Québec en Outaouais (UQO), Ripon, Quebec, Canada

In preparation

Key words: Carbohydrate reserves, functional traits, carbon investment strategies, tropical trees, temperate trees

2.1 ABSTRACT

Carbohydrate reserves play a vital role in plant survival during periods of negative carbon balance. Thus, at the plant level, it is expected that carbon allocation to carbohydrate reserves exhibits a coordinated variation with a suite of functional traits that are related to acquisition and conservation of carbon resources.

To test the relationship between plant functional economic traits and allocation to reserves, we sampled 80 tree species from temperate and tropical forests. We evaluated non-structural carbohydrates (NSC) and 16 traits (including specific leaf area, photosynthetic capacity, wood density, leaf nutrients, and height).

The relationship between functional traits and carbohydrate concentrations was orthogonal. The first axis was formed by traits that define the leaf and wood economics spectrum and the second axis was defined by NSC concentrations. Except for a significant relationship between carbohydrate concentrations in roots and tree height, most of the relationships between NSC concentrations in woody tissues and traits were weak or non-significant.

Investment in traits that were associated with resource conservation is not related to investment in NSC storage. These results provide new insights about the allocation of carbon to storage or defenses in trees with different life strategies.

2.2 INTRODUCTION

Carbohydrate reserves, mainly non-structural carbohydrates (NSC) comprised of sugar and starch (Hoch, Richter & Körner 2003), play a vital role in plant survival during periods of negative carbon balance induced by light limitation (Myers & Kitajima 2007; Poorter & Kitajima 2007), drought (McDowell *et al.* 2008; Mitchell *et al.* 2013; O'Brien *et al.* 2014), cold temperatures (Handa, Körner & Hättenschwiler 2005; Hoch & Körner 2012; Fajardo & Piper 2014; Piper *et al.* 2016), diseases, and physical damage due to herbivory and falling debris (Kobe 1997; Canham *et al.* 1999; Myers & Kitajima 2007; Atkinson *et al.* 2014). Additionally, in temperate and boreal plants, a higher proportion of net carbon assimilation is allocated to NSC to provide the energy to maintain respiration during long winters and for vegetative growth in spring (Gaucher *et al.* 2005; Gough *et al.* 2009; Messier *et al.* 2009).

At the plant level, the allocation of carbon to reserves has been hypothesized as a competition with growth and other physiological processes such as defense (Chapin, Schulze & Mooney 1990; Sala, Woodruff & Meinzer 2012; Dietze *et al.* 2013), which suggests a trade-off between carbon allocated to growth and that allocated to reserves and defense (Kitajima 1994; Kobe 1997; Myers & Kitajima 2007). Carbon allocation to growth, in turn, is involved in other trade-offs that are related to the 'fast-slow' plant economics (Reich 2014) and size spectra (Grime *et al.* 1997; Westoby *et al.* 2002; Diaz *et al.* 2004; Wright *et al.* 2004; Chave *et al.* 2009; Reich 2014; Díaz *et al.* 2016).

These suites of trade-offs reflect a coordinated variation among plant traits from species that differ in growth form, size, phylogeny, and biome (Reich, Walters & Ellsworth 1997; Reich *et al.* 1999; Wright *et al.* 2004; Donovan *et al.* 2011; Reich 2014; Díaz *et al.* 2016). For example, fast-growing, resource-acquisitive species, are

generally characterized by high specific leaf area (SLA), high leaf nutrient concentrations, and low wood density. On the contrary, slow-growing, resource-conservative species, are generally characterized by low SLA, low leaf nutrient concentrations, and high wood density (Reich *et al.* 1998; Wright *et al.* 2004; Poorter & Bongers 2006; Baraloto *et al.* 2010; Wright *et al.* 2010).

Although intra- and inter-specific variation of NSC concentrations may be caused by multiple factors, e.g., climate, soil, biotic interactions, phylogeny, it is expected that carbon allocation to reserves exhibits a coordinated variation with a suite of functional traits that are related to resource acquisition and conservation. For instance, a higher SLA indicates a higher light capture potential, a higher net photosynthetic rate, and higher concentrations of nutrients such as N in leaves (Wright, Westoby & Reich 2002; Wright *et al.* 2004). Therefore, it is expected that an increase in SLA leads to an increase in carbohydrate reserves in certain plant organs, such as leaves, as a result of higher photosynthetic rates (Li *et al.* 2016). In addition, tough leaves and dense woody tissues suggest greater carbon investment in defense traits to resist and to recover from biotic and abiotic stress (Poorter & Kitajima 2007; Poorter *et al.* 2010), which co-vary with carbon allocation to reserves, especially in roots (Kitajima 1994; Myers & Kitajima 2007).

Furthermore, other traits, such as tree height, might be related to carbon allocation to reserves. NSC concentrations in woody tissues generally increase with plant height (Genet, Bréda & Dufrêne 2009; Sala & Hoch 2009; Piper & Fajardo 2011; Woodruff & Meinzer 2011). High concentrations of NSC are suggested to be necessary to maintain safety margins due to a reduced hydraulic efficiency associated with increasing tree height (Sala, Woodruff & Meinzer 2012) or the higher risk of stem breakage (Niklas 1992). Together, these findings suggest that variation in NSC

concentrations may be jointly constrained by that of leaf and wood traits and tree height.

To date, there have not been any investigations of large-scale ecological patterns of variation between plant functional traits and allocation to reserves. To better understand these relationships, we sampled 80 tree species from temperate deciduous, upper montane tropical, and lowland tropical forests. Because allocation to reserves and other major functional traits are critical to survival, we expected that (*i*) there would be a coordinated variation between NSC concentrations and the leaf and wood economic spectra, independent of biomes; (*ii*) that species with higher NSC concentrations would have trait values associated with resource conservation or 'slow' ecological strategies, such as a low SLA, high tissue density, and low concentrations of leaf nutrients, and vice versa for species with trait values associated with resource acquisition or 'fast' ecological strategies; and (*iii*) that tree height would scale positively with concentration of reserves, because tree height determines changes in functional traits when plants experience increased light levels.

2.3 METHODS

2.3.1 Research sites

This study was carried out in a deciduous temperate forest (DTF; Mont St-Hilaire, Quebec, Canada) and in an upper montane forest (UMF) and a lowland tropical forest (LTF) in Colombia (Table 2.1). Sites were selected to obtain contrasts in latitude and seasonality (Canada versus Colombia) and altitude (within Colombia). Study sites had not experienced recent anthropogenic disturbances at the time of sampling.

Table 2.1 Main characteristics of the study sites.

	Rio Claro Reserve, Antioquia, Colombia (LTF)	**Hacienda Sabaneta Nature Reserve. Cundinamarca, Colombia (UMF)**	**Gault Nature Reserve. Mont-Saint-Hilaire, Quebec, Canada (DTF)**
Biome	Lowland tropical rainforest	Upper montane forest	Deciduous temperate forest
Latitude	5°54'04'' N	4°32'30'' N	45°32'31'' N
Longitude	74°51'24'' W	74°15'18'' W	73°09'11'' W
Altitude (range, m asl)	250 - 750	2500 - 3300	200 - 400
Mean annual precipitation (mm)	4000	1900	967
Mean annual temperature (°C)	26	12	6 (16*)
Mean annual freeze-free days	NA	NA	140
Number of species studied	32	27	21

* Mean growing season temperature.

2.3.2 Field sampling

We sampled a total of 80 native tree species (Annex 1) across the three study sites in 2012. In the temperate forest, samples were taken after bud break and hardening (May) and at the end of the growing season (October) to capture some of the seasonal dynamics in carbon reserves that are typical of northern temperate forests. In the tropical forest sites, samples were taken during the transition from the dry to rainy seasons (January to April). At each site, the most abundant tree species were selected for sampling. Botanical samples of tropical species were taken for verification and deposited at the Medellin Botanical Garden. At the temperate forest site, species identification had been validated previously.

Leaves and wood (including tissues from branches, stems, and roots) were sampled from 3-5 individuals for each species. In total, we collected 1271 samples from trees. Young, fully expanded leaves from adult plants without visible symptoms of pathogen or herbivore attack were sampled at the top of the tree. To avoid possible effects of diurnal variation in NSC, leaf samples were collected in the early morning (Upmeyer & Koller 1973). Stem samples were taken with a 4.3 mm diameter increment borer. Stem cores were taken perpendicular to the slope to reduce variability in wood density due to compression or tension. Samples of top branches 2-3 cm in diameter were obtained by cutting them down with a tree trimmer. Root samples were taken with an increment borer from large surface roots ca. 50 cm away from the base of the stem.

2.3.3 Functional traits

We measured 16 traits that were associated with important ecological strategies for tree functioning, productivity, and survival (Table 2.2) following standard protocol (Pérez-Harguindeguy *et al.* 2013).

Table 2.2 List of functional traits considered in this study with the abbreviations used in the text, the units of expression, and the ecological role of the trait.

Parameter	Abbreviation	Units	Ecological role
Leaf size	LS	mm^2	Resource acquisition
Leaf thickness	LT	mm	Resource acquisition and defense
Leaf dry matter content	LDMC	$mg\ g^{-1}$	Resource acquisition and defense
Specific leaf area	SLA	$mm^2\ mg^{-1}$	Resource acquisition and defense
Photosynthetic capacity (area base)	Amax_area	$\mu mol\ CO_2\ m^{-2}\ s^{-1}$	Resource acquisition
Photosynthetic capacity (mass base)	Amax_mass	$nmol\ CO_2\ g^{-1}\ s^{-1}$	Resource acquisition
Foliar carbon	C	%	Resource acquisition and defense
Foliar nitrogen	N	%	Resource acquisition and defense
Foliar phosphorus	P	$mg\ kg^{-1}$	Resource acquisition
Foliar potassium	K	$mg\ kg^{-1}$	Resource acquisition
Foliar calcium	Ca	$mg\ kg^{-1}$	Resource defense
Foliar magnesium	Mg	$mg\ kg^{-1}$	Resource acquisition and defense
Stem density	SD	$mg\ mm^{-3}$	Hydraulic transport, mechanical strength and defence
Branch density	BD	$mg\ mm^{-3}$	Hydraulic transport, mechanical strength and defence
Root density	RD	$mg\ mm^{-3}$	Hydraulic transport, mechanical strength and defence
Tree height	H	m	Resource capture and reproduction
Foliar sugars	Sugar_L	%	Carbon and energy source

Parameter	Abbreviation	Units	Ecological role
Foliar starch	Starch_L	%	Carbon and energy source
Foliar total NSC	NSC_L	%	Carbon and energy source
Stem sugars	Sugar_S	%	Carbon and energy source
Stem starch	Starch_S	%	Carbon and energy source
Stem total NSC	NSC_S	%	Carbon and energy source
Branch sugars	Sugar_B	%	Carbon and energy source
Branch starch	Starch_B	%	Carbon and energy source
Branch total NSC	NSC_B	%	Carbon and energy source
Root sugars	Sugar_R	%	Carbon and energy source
Root starch	Starch_R	%	Carbon and energy source
Root total NSC	NSC_R	%	Carbon and energy source

Leaf size (LS, mm^2), leaf thickness (LT, mm), leaf dry matter content (LDMC, mg g^{-1}), and specific leaf area (SLA, mm^2 mg^{-1}): Eight completely expanded leaves from each individual were collected from the sampled branch. Leaves were placed in plastic bags in the field with damp paper to maintain humidity. LS was measured using WinFolia software (*Regent Instruments, Toronto, Canada*). LT was measured in fresh leaves as the mean of four measurements with a digital micrometer (Mitutoyo Instruments, Singapore). LDMC was calculated as the leaf dry mass at 60 °C divided by its water-saturated fresh mass. SLA was calculated as the area of the fresh lamina surface divided by its dry mass.

Photosynthetic capacity by mass (Amax_mass: nmol CO_2 g^{-1} s^{-1}) and area (Amax_area: µmol CO_2 m^{-2} s^{-1}): Photosynthetic capacity was measured on leaves from two branches in both tropical forest sites using a LiCor model 6400 portable photosynthetic system (LiCor, Lincoln, NE, USA). The photosynthetic capacity under saturating light (A_{max}) was measured at 2000 µmol m^{-2} s^{-1}. Measurements were carried out under constant CO_2 concentration (390 ppm) and leaf temperature (set at 20 °C). Leaves were allowed to acclimate to 1000 µmol m^{-2} s^{-1} and then 2000 µmol m^{-2} s^{-1} for 5 min before measurements. Photosynthetic capacity per leaf dry mass was calculated as the product of Amax_area and SLA^{-1}. Photosynthetic data from Mont St. Hilaire trees were taken from Marino, Aqil and Shipley (2010).

Analysis of carbon (C, %), nitrogen (N, %), and nutrients (Ca, K, Mg, and P, mg kg^{-1}): Leaf tissues for these analyses were dried and ground to a fine powder using a ball mill. C and N concentrations were determined for leaf samples from all trees with a CN analyzer (Elementar Vario Max). Determination of Ca, K, Mg, and P was performed on 100 samples using the acid digest method (Allen 1974), and then these results were extrapolated to all samples of tree leaves with a near-infrared

spectroscopy (NIRS) model (Ramirez *et al.* 2015). C, N, and nutrient analyses were performed at the Max Planck Institute for Biogeochemistry in Jena, Germany.

Wood density (stem density (SD), branch density (BD), and root density (RD), mg mm^{-3}): Samples of roots, stems, and branches were placed in plastic bags in the field with damp paper to maintain humidity, and then they were soaked in water in the lab for 48 hours. Fresh volume was measured by water displacement, and wood mass was determined after drying samples at 60 °C to a constant weight and then again at 100 °C to a constant weight (Williamson & Wiemann 2010).

Tree height (H, m): Tree height was measured on each tree sampled with a TruPulse 360 laser (Laser Technology, Inc., CO, USA). The device resolution is 10 cm for linear lengths.

Non-structural carbohydrates (sugar, starch, and NSC, % of dry matter): Leaves and wood samples for NSC analysis were placed in paper bags and refrigerated. These samples were then microwaved in the lab within 8 h after sampling to stop enzymatic activity (Popp *et al.* 1996). Leaf samples were ground using a ball mill and wood samples were ground using a coffee grinder with a mesh sieve. A sub-sample of 180 (of a total of 1271) was selected using the Kennard–Stone algorithm (Kennard & Stone 1969) for NSC analysis following (Hoch, Popp & Körner 2002). Ground plant material was dissolved for 30 min in distilled water. Starch and sucrose were disaggregated in glucose and in glucose and fructose, respectively, with Clarase (*Aspergillus oryzae*, Enzyme Solutions Pty Ltd, Crydon South, Victoria, Australia) by incubation at 40°C for 15 h. Phosphoglucose-isomerase was added to the solution and then the total amount of glucose (corresponding to total NSC) was quantified photometrically in a microplate photometer at 340 nm (Thermo Fisher Scientific, Waltham, USA) after conversion of glucose to gluconate-6-phosphate (hexokinase; Sigma-

Aldrich, St. Louis, MO, USA). An aliquot of the original extract was treated with invertase and phosphoglucose-isomerase (both Sigma-Aldrich) to determine the amount of glucose, fructose, and sucrose using a glucose test (see above). Starch was calculated as NSC minus sugar. Pure starch and glucose, fructose, and sucrose solutions were used as standards. Plant powder from orchard leaves (Leco, St. Joseph, MI, USA) was used as a standard reference material. The NSC concentrations are reported here as a percentage of dry matter. Sugar, starch, and NSC values were extrapolated to all samples using near-infrared reflectance spectra. Reflectance spectra were measured using a FT-NIR Analyzer (Bruker MPA Multi-Purpose FT-NIR Analyzer) for all samples. The reflectance spectra were taken from 800 to 2780 nm with a mean spectral resolution of 1.7 nm on 5 scans per sample. The spectral data were recorded as absorbances (log 1/R, where R = reflectance). Regression models that predict carbohydrate concentrations in different plant tissues (leaves, stems, branches, and roots) from near-infrared reflectance spectra were developed using partial least squares regression and competitive adaptive re-weighted sampling. The adjustment of the models obtained was $r^2 = 0.91$, $r^2 = 0.85$, and $r^2 = 0.82$ for NSC, starch and sugars, respectively (Ramirez *et al.* 2015).

2.3.4 Statistical analysis

To avoid multicollinearity among traits, we evaluated correlations among each pair of predictor traits. Traits with a Spearman's correlation value greater than 0.70 were filtered out using the findCorrelation function of the 'caret' package (Kuhn 2008). This process led to an elimination of four collinear predictors (BD, P, Ca and C), yielding a final 12 traits used for further analysis.

The relationship between the group of traits (i.e. leaf, stem, or root) and the NSC concentrations in plant tissues was evaluated using multiple factor analysis (MFA).

This multivariate technique identifies the common structure present in the datasets defined for the same group of individuals (Escofier & Pages 1994). To evaluate the degree of coordination between traits and NSC, we used the RV coefficient, which is a coefficient between 0 and 1 that indicates the relationship between the two sets of traits and the contribution of the variables to the components. This analysis was performed with the 'FactoMineR' package (Lê, Josse & Husson 2008). To analyze bivariate relationship between traits and NSC concentrations, we used standardized major axis (SMA) analyses. SMA determines how carbohydrate reserves in the different tissues scale with functional traits and how this relationship changes across biomes. To compare SMA lines among biomes, we tested if the relationship under consideration had a slope that was different from zero (Warton *et al.* 2006). Finally, to compare common-slopes between biomes a pairwise comparison of biome slopes was performed. SMA tests were performed with the 'smatr' R package (Warton *et al.* 2012).

Finally, linear mixed-effect models were used to test the extent to which height influenced the scaling of NSC with traits. The models predicted carbohydrate concentrations (sugar, starch, and NSC) in the different tissues (leaves, roots, stems, and branches) as a function of traits, height, and site. The model included species as a random effect. Models were evaluated using analysis of variance (ANOVA) with the 'LMERConvenienceFunctions' R package (Tremblay 2012). Models were simplified by a backward selection of fixed effects. Model terms were removed at a threshold of 0.05 and then a new model was fitted. Also, the complex and the simplified models were compared by a log-likelihood ratio test. Finally, collinearity effects on the overall model were assessed using the variance inflation factor (VIF) and the kappa statistic for collinearity. Overall, variables with VIF values higher than 5 and models with a kappa higher than 30 were dropped and recalculated until values below these thresholds were achieved.

Carbohydrate and trait data were $\log_{10}$ transformed to meet assumptions of normality and homogeneity of variance. All statistical analyses were conducted in R v. 3.02 (R Foundation for Statistical Computing, Vienna, Austria).

2.4 RESULTS

2.4.1 Traits and variability in carbohydrate concentrations across biomes

Mean concentrations of NSC were higher in roots followed by leaves, branches, and stems (Figure 2.1). Mean NSC concentrations in leaves of broadleaved temperate trees were significantly higher than in tropical trees. On the contrary, NSC concentrations in temperate trees were significantly lower in stems and similar in branches and roots compared with trees from the tropical lowland and montane biomes (Figure 2.1). Mean values of LDMC, SLA, Amax_mass, and height were higher in temperate than in tropical trees, but leaf size, leaf thickness, Amax_area, K, and Mg were lower (Figure 2.1). Mean values of leaf N and wood density (SD and RD) were similar among biomes. The mean concentrations of NSC differed significantly among tissues ($p < 0.05$).

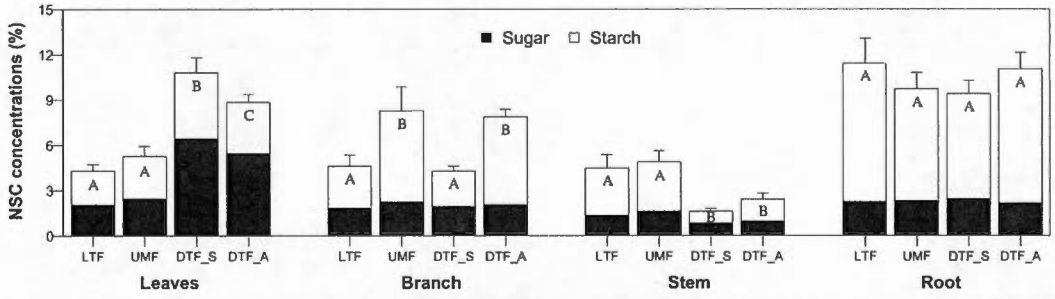

Figure 2.1 Mean concentrations of carbohydrates (with standard error) in tree tissues from lowland tropical rainforest (LTF).

Upper montane forest (UMF), and deciduous temperate forest (DTF). Concentrations of carbohydrates for DTF are divided in spring (S) and autumn (A). Different letters indicate significant differences in mean values among biomes (Tukey's tests, α = 0.05).

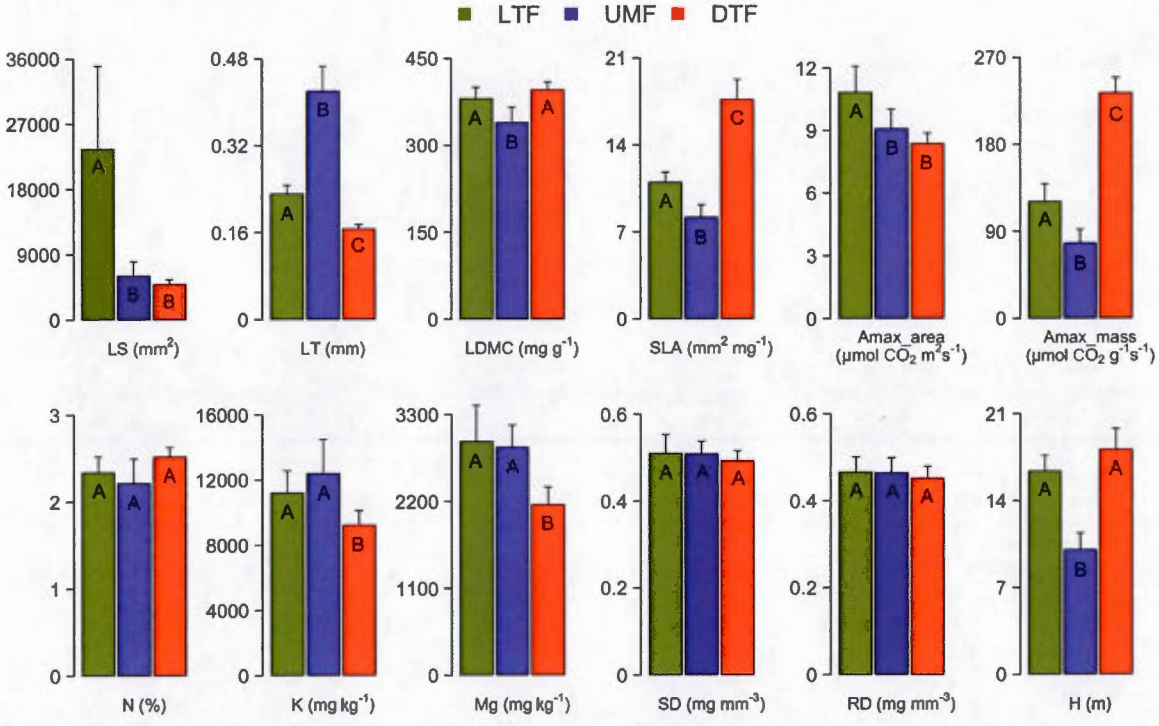

Figure 2.2 Mean trait values (with standard error) in trees from lowland tropical rainforest (LTF), upper montane forest (UMF), and deciduous temperate forest (DTF). Different letters indicate significantly different mean values among biomes (Tukey's tests, $\alpha = 0$.

2.4.2 Relationships between carbohydrate concentrations and functional traits

Overall, the relationship between functional traits measured and the carbohydrate concentrations was orthogonal, which suggested little coordination between these variables. Conversely, the relationship between both groups was not orthogonal in the DTF (Figure 2.3). The RV coefficient of the MFA showed a significant relationship between traits and carbohydrates in the DTF (RV = 0.18, $p < 0.05$), but not in LTF and UMF. The contribution of the variables to the axis of the MFA analysis are presented in Annex 2.

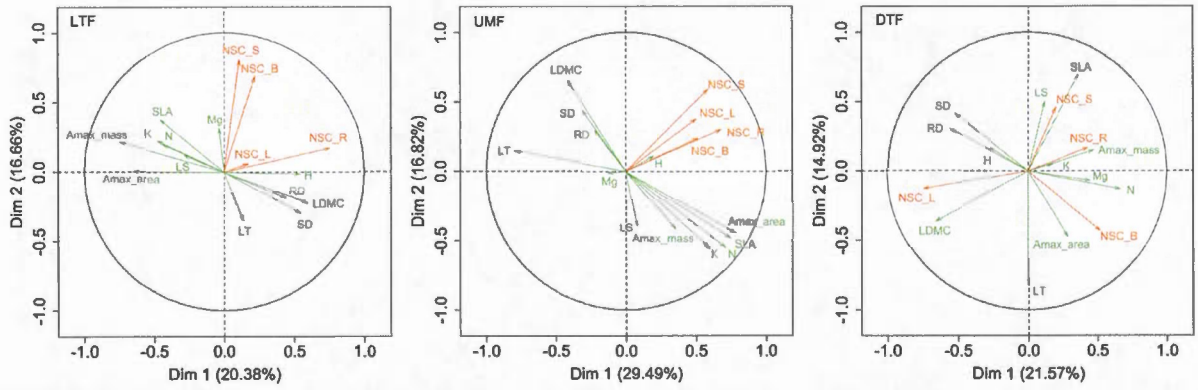

Figure 2.3 Multiple factor analysis of functional traits (green) and NSC concentrations (brown) in tree tissues from lowland tropical rainforest (LTF).

Upper montane forest (UMF), and deciduous temperate forest (DTF). Refer to Table 2.2 for trait abbreviations

The pairwise relationships of leaf traits and NSC in leaves (especially sugar) were strongly correlated, reaching *r* values up to 0.5 (Table 2.3). Species with higher carbohydrate concentrations in leaves had lower LT, Amax_area, and Mg, and higher SLA, Amax_mass, and N (Table 2.3). In contrast, traits and carbohydrates in woody tissues were either significantly correlated with low *r* values or were not significantly correlated. Taller trees tended to have smaller sugar and NSC concentrations in stems and larger concentrations in roots and leaves (Table 2.3). Nevertheless, biome-specific relationships were mostly not significant. In several cases, the relationships presented a contrasting patterns of variation among biomes, indicated by the low number of relationships with common slopes (Fig 4 and Annex 3). For example, the relationships between NSC in roots and Amax_mass in both tropical biomes did not exhibit similar slopes (Fig 4 and Annex 3).

Table 2.3 Correlation coefficients of the relationship between functional traits and carbohydrate concentrations.

	LS	LT	LDMC	SLA	Amax_area	Amax_mass	N	K	Mg	SD	RD	H
Sugar_R	0.06	-0.10	**0.16**	0.01	0.14	**0.18**	0.01	-0.05	0.11	-0.13	**-0.21**	0.08
Starch_R	0.00	**-0.30**	0.09	**0.19**	-0.02	**0.18**	0.13	0.01	-0.04	0.11	0.09	**0.29**
NSC_R	-0.02	**-0.29**	0.12	**0.22**	-0.09	**0.20**	**0.18**	0.06	-0.04	0.09	0.05	**0.28**
Sugar_S	-0.09	**0.26**	-0.18	**-0.21**	-0.04	**-0.23**	0.00	0.14	**0.20**	-0.03	-0.11	**-0.21**
Starch_S	-0.15	0.10	-0.12	0.00	-0.07	**-0.21**	0.14	0.01	**0.19**	-0.06	-0.07	-0.14
NSC_S	-0.12	0.11	**-0.18**	-0.05	-0.11	**-0.26**	0.13	0.09	**0.35**	-0.02	-0.01	**-0.19**
Sugar_B	0.14	**0.18**	-0.14	-0.15	-0.11	-0.14	-0.06	**0.18**	**0.21**	-0.08	-0.10	-0.15
Starch_B	-0.07	-0.11	0.04	0.10	-0.11	0.09	**0.19**	0.11	0.02	-0.05	-0.09	0.04
NSC_B	0.09	-0.07	-0.07	0.09	0.01	0.12	**0.20**	**0.24**	0.07	-0.11	-0.10	0.03
Sugar_L	**-0.26**	**-0.51**	**0.25**	**0.45**	-0.11	**0.47**	0.13	**-0.21**	**-0.28**	0.07	0.04	**0.32**
Starch_L	0.00	**-0.18**	-0.06	**0.24**	**-0.20**	**0.20**	0.10	0.08	-0.05	0.14	0.06	0.06
NSC_L	-0.13	**-0.52**	0.13	**0.49**	**-0.23**	**0.46**	**0.18**	-0.11	**-0.20**	0.09	0.04	**0.20**

Values in bold express significant correlations ($p < 0.05$). Refer to Table 2.2 for trait abbreviations.

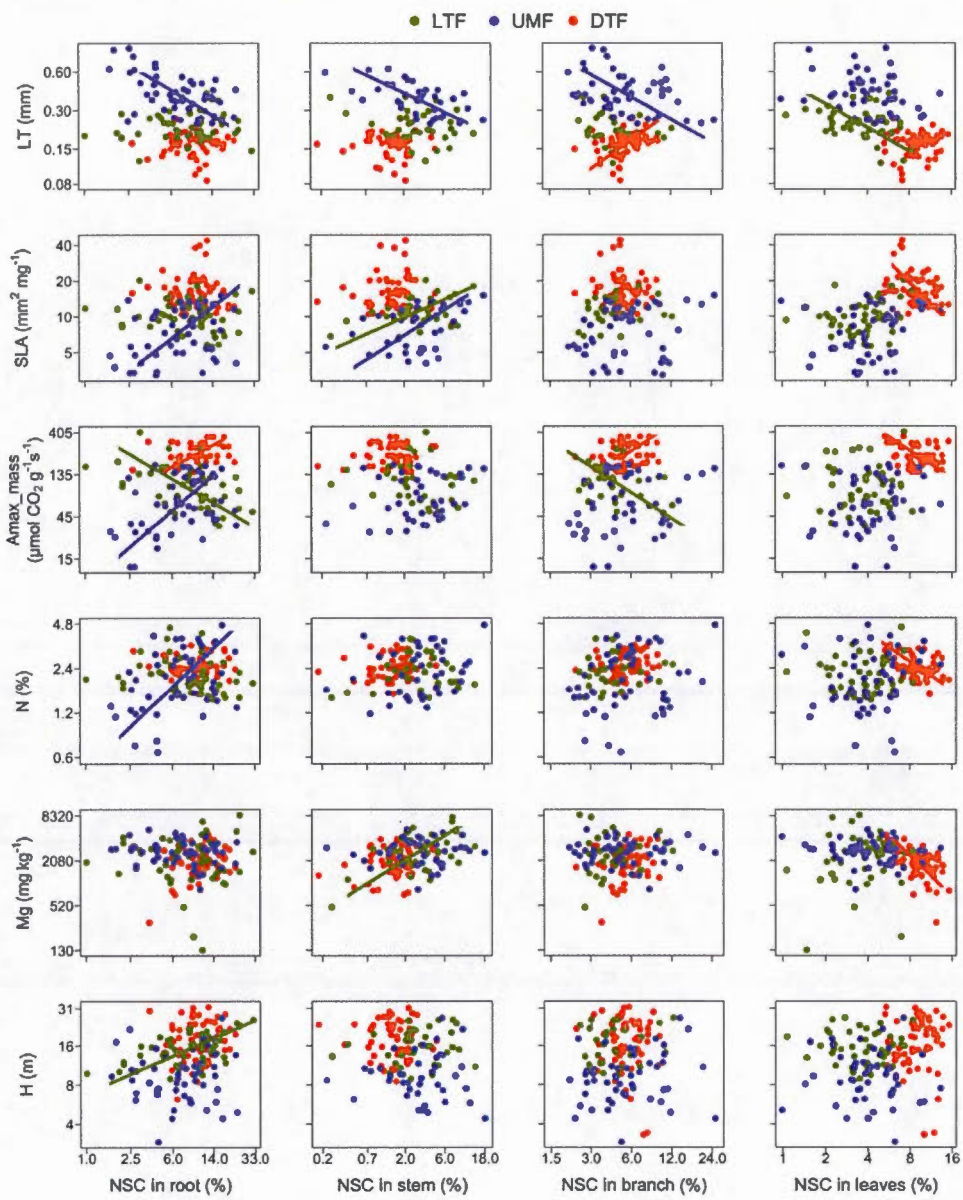

Figure 2.4 Correlations between NSC concentrations and functional traits in trees from upper montane forest (UMF), lowland tropical rainforest (LTR), and deciduous temperate forest (DTF).

Lines represent significant bivariate relationships for all trees (gray) or per biome (colors) ($p < 0.05$). Refer to Table 2.2 for trait abbreviations. Axes are in log scale

2.4.3 Height - NSC relationships

Linear mixed-effect models showed that height (H) predicted and scaled positively with the NSC concentrations in roots. This effect was not inter-dependent of traits or biome with the exception of LT, which had a significant effect (Table 2.4). For NSC concentrations in stems and leaves, site had a significant effect in all models that we evaluated whereas H was not significant. This indicated that those significant correlations between NSC concentrations in stems and leaves (Table 2.3) were probably an indirect effect, because trees in DTF were taller than those in the LTF and UMF. None of the interactions between traits and H were statistically significant, which indicated that the response of NSC concentrations did not depend on a covariation with height (in the case of roots) or site (in the case of stem and leaves).

Table 2.4 Fixed-effect estimated parameters for NSC concentrations in leaves and woody tissues in relation to traits, height, and site. Values in bold represent significant parameters ($p < 0.05$).

Model: NSC ~	Roots				Stem				Branch				Leaves			
	Trait	H	Site	Trait*H	Trait	H	Site	Trait*H	Trait	H	Site	Trait*H	Trait	H	Site	Trait* H
LS * H * site	-0.02 ± 0.06	**0.24 ± 0.12**	-0.09 ± 0.19				**0.63 ± 0.21**		0.07 ± 0.05		**-0.26 ± 0.14**				**-0.9 ± 0.12**	
LT * H * site	**-0.36 ± 0.19**	**0.25 ± 0.12**	0.01 ± 0.19		-0.59 ± 0.23		**0.90 ± 0.20**			0.03 ± 0.09	-0.21 ± 0.14		**-0.27 ± 0.14**		**-0.82 ± 0.12**	
LDMC * H * site		**0.25 ± 0.12**	-0.11 ± 0.18		0.04 ± 0.37	-0.12 ± 0.17	**0.71 ± 0.21**				-0.21 ± 0.14		-0.03 ± 0.20		**-0.91 ± 0.12**	
SLA * H * site		**0.25 ± 0.12**	-0.10 ± 0.18		0.50 ± 0.19		**0.83 ± 0.21**				-0.21 ± 0.14		**0.12 ± 0.11**		**-0.85 ± 0.13**	
Amax_area * H * site	-0.15 ± 0.14	**0.24 ± 0.12**	-0.09 ± 0.18		-0.37 ± 0.18		**0.70 ± 0.20**				-0.21 ± 0.14				**-0.87 ± 0.12**	
Amax_mass * H * site	-0.02 ± 0.11	**0.25 ± 0.12**	-0.13 ± 0.21		**0.01 ± 0.17**	-0.11 ± 0.17	**0.71 ± 0.25**						**0.04 ± 0.08**		**-0.87 ± 0.13**	
N * H * site	0.05 ± 0.19	**0.25 ± 0.12**	-0.10 ± 0.19		0.80 ± 0.50		**0.71 ± 0.21**	-0.24 ± 0.18			-0.21 ± 0.14		0.03 ± 0.14		**-0.90 ± 0.12**	
K * H * site	-0.07 ± 0.08	**0.24 ± 0.12**	-0.1 ± 0.19				**0.63 ± 0.21**				-0.21 ± 0.14		-0.05 ± 0.06	-0.05 ± 0.09	**-0.90 ± 0.12**	
Mg * H * site	-0.06 ± 0.09	**0.25 ± 0.12**	-0.10 ± 0.19		**0.26 ± 0.15**		**0.55 ± 0.21**				-0.21 ± 0.14		0.02 ± 0.08		**-0.91 ± 0.12**	-0.01 ± 0.01
SD * H * site	0.32 ± 0.20	**0.23 ± 0.12**	-0.11 ± 0.18				**0.64 ± 0.21**		-0.12 ± 0.18	0.04 ± 0.09	-0.21 ± 0.14		0.14 ± 0.30		**-0.91 ± 0.12**	0.02 ± 0.11

RD * H * site	0.14 ± 0.19	**0.24 ± 0.12**	-0.11 ± 0.18	**0.64 ± 0.21**	0.04 ± 0.09	-0.2 ± 0.14	0.01 ± 0.14	**-0.91 ± 0.12**

Refer to Table 2 for trait abbreviations.

2.5 DISCUSSION

Our results describe the relationships between the allocation of carbon to reserves and numerous functional traits of temperate and tropical tree species. We found coordinated variation between NSC concentrations and most traits in leaves (Table 2.3, Figure 2.4). However, relationships between traits and NSC in woody tissues were weak or non-significant. Additionally, we identified a relationship between carbohydrate concentrations in roots and tree height that may provide insights about the plant survival strategies across biomes.

2.5.1 Relationships between carbohydrate concentrations and functional traits

Partially supporting our first hypothesis, we found strong covariation between leaf functional traits and carbohydrates in leaves (Figure 2.3, Table 2.3). The strong relationship observed between functional traits and carbohydrate reserves in leaves of DTF species provides evidence of an acclimation strategy to maintain metabolic activity in colder environments by increasing the storage of NSC and N and increasing SLA and Amax (Tjoelker, Reich & Oleksyn 1999; Campbell *et al.* 2007; Xiang *et al.* 2013). In addition, leaf structure of short-lived leaves of temperate-deciduous trees with lower SLA and high Amax_max indicates that they contain tightly packed cells of palisade parenchyma in which NSC are stored (Poorter *et al.* 2009), and thus likely more carbon that can be allocated to reserves. The apparent trait-mediated constraints on carbohydrates in leaves may allow deciduous trees to rapidly accumulate reserves for use in the dormant season and for bud break the following growing season (Kramer & Kozlowski 1979).

Relationships between functional traits and carbohydrate concentrations in leaves (in LTF and UMF), stems, roots, and branches were either weak or not statistically significant. Our results clearly delineate two orthogonal axes of variation (Figure 2.3,

Table 2.3). The first axis was formed by traits that define the leaf and wood economics spectrum (Wright *et al.* 2004; Chave *et al.* 2009) and the second axis was defined by NSC concentrations. Our results suggest that functional traits and carbohydrate concentrations in the studied tropical biomes exhibited orthogonal strategies for plant carbon acquisition and conservation. That is, species with a conservative carbon acquisition strategy, i.e., shade-tolerant species, may be characterized by a proportionally large allocation of carbon to reserves and have conservative functional traits (Kobe 1997; Myers & Kitajima 2007; Poorter & Kitajima 2007; Atkinson *et al.* 2012). However, our results suggested that the lack of coordination between functional traits and carbohydrate concentrates may not always hold, particularly in large trees. Other studies have also reported that there was no trade-off between allocation of carbohydrates to reserves and carbon investment to conservative functional traits, such as those that improve survival in low-light (Lusk & Piper 2007; Imaji & Seiwa 2010; Piper 2015).

We had expected that functional traits and NSC concentrations would scale with similar slopes among biomes (Wright *et al.* 2004; Wright *et al.* 2005a; Reich 2014). Because traits varied independently of NSC concentrations, we found few significant relationships and, among those that were significant, contrasting trends in the relationships among the three biomes studied (Figure 2.4, Annex 3). Several other studies on woody plants have reported contrasting trade-off correlations among floras (Wright *et al.* 2005b; Heberling & Fridley 2012; Heberling & Fridley 2013). Contrasting patterns of plant functional strategies among plant communities have been associated with phylogenetic constraints, or selective biogeographic processes, such as adaptation to different climatic regimes or physical barriers that generate different selective pressures within communities.

The orthogonal relationship between NSC concentrations and functional traits may be because carbon accumulation and the leaf economic spectrum act on different time scales. Allocation of reserves to pools such as branches, stems, or roots operate over several seasons or even years, depending on the distance and the osmotic gradient between carbohydrate sources and sinks (Lacointe 2000; Hartmann & Trumbore 2016). Overall, NSC reserves that have been stored recently in leaves and branches support daily metabolism and annual growth, but older reserves stored in stems and roots may contribute to regrowth after disturbance (Vargas, Trumbore & Allen 2009; Carbone *et al.* 2013; Richardson *et al.* 2013). The lower mean NSC concentrations that we found in spring in branches of DTF indicated that the carbohydrates for leaf flush were supplied from the closest sources of reserves (branches) and not from the more distant ones with older reserves (stems and roots) that remained with similar concentrations (Schädel *et al.* 2009; Hoch 2015). Thus, NSC stored in stems and roots probably remain sequestered and may not vary considerably until a disturbance triggers an imbalance between carbon sources and sinks and initiates mobilization of reserves. In addition, trees under normal function (not facing large disturbances or stress) may store a great amount of carbohydrate reserves, which in some cases would be enough to rebuild the whole leaf canopy up to four times (Hoch, Richter & Körner 2003; Körner 2003; Würth *et al.* 2005), and the whole carbon pool in temperate broadleaved trees were estimated to store enough carbon to supply stem growth for up to 30 years (Klein, Vitasse & Hoch 2016).

A factor that may influence the relationship between traits and NSC concentrations in leaves is that, independent of whether a species is 'conservative' or 'acquisitive' in terms of carbon use, species can invest carbon differentially between storage and defenses to maximize survival and/or growth (Coley, Bryant & Chapin 1985; Kobe 1997). In general, leaf damage by herbivory and pathogens in tropical forests is higher and more costly (due to leaf life span) than in deciduous temperate forests

(Coley & Barone 1996). Hence, tropical species may preferentially invest more carbon in defenses than in carbohydrate reserves and, in this way, reduce the relationship between structural leaf traits and NSC concentrations.

2.5.2 Height relationship with NSC concentrations

Partially supporting our first hypothesis, height (H) scaled positively with NSC concentrations in roots, but it did not co-vary with traits and site (Table 2.4). This result may be explained by the increased surplus of carbon due to the lower carbon demand for structural growth, defense, and reproduction as trees grow (Ryan, Binkley & Fownes 1997). In addition, other possible factors that may explain the correlation between height and NSC concentrations include constraints on phloem transport in taller trees, which reduce the transport of carbohydrates from the carbon pools to the sinks, resulting in the accumulation of NSC in roots (Woodruff & Meinzer 2011). Also, because taller trees tend to have higher amounts of parenchyma than smaller trees (Ziemińska, Westoby & Wright 2015), they may also have a higher capacity to store carbohydrates (Morris *et al.* 2016). Finally, the higher concentration in roots may serve as long-term storage to respond to future stresses common to taller trees, such as physical damage from falling trees, branches, and litter (Clark & Clark 1991; Clarke *et al.* 2013).

2.6 ACKNOWLEDGEMENTS

This study would not have been possible without support from Milena Molina, Juan Carlos Medina, Luis Carlos Galeano, David Andres Herrera, Sergio Martinez, and Mathieu Messier. We also thank Alfredo Navas (Hacienda Sabaneta Natural Reserve) and Alvaro Cogollo (Medellin Botanical Garden) for their logistical support and Thomas Gavin for language editing. This research was supported by the NSERC/Hydro-Quebec research chair on tree growth control and by a scholarship from the Quebec research funds for nature and technology. We would like to thank the anonymous reviewers for their very helpful comments on the manuscript.

2.7 SUPPORTING INFORMATION

2.7.1 Annex A

Table 2.5A List of tree species sampled in Colombia and Canada.

UMF: upper montane forest, LTF: lowland tropical rainforest, and DTF: deciduous temperate forest.

Family	Genus	Species	Site
Sapindaceae	*Acer*	*pensylvanicum*	DTF
Sapindaceae	*Acer*	*rubrum*	DTF
Sapindaceae	*Acer*	*saccharinum*	DTF
Sapindaceae	*Acer*	*saccharum*	DTF
Lamiaceae	*Aegiphila*	*bogotensis*	UMF
Betulaceae	*Alnus*	*acuminata*	UMF
Betulaceae	*Alnus*	*incana*	DTF
Lauraceae	*Aniba*	*perutilis*	LTF
Malvaceae	*Apeiba*	*glabra*	LTF
Olacaceae	*Aptandra*	*tubicina*	LTF
Apocynaceae	*Aspidosperma*	*megalocarpon*	LTF
Ericaceae	*Bejaria*	*resinosa*	UMF
Melastomataceae	*Bellucia*	*pentamera*	LTF
Betulaceae	*Betula*	*alleghaniensis*	DTF
Betulaceae	*Betula*	*papyrifera*	DTF
Moraceae	*Brosimum*	*utile*	LTF
Lecythidaceae	*Cariniana*	*pyriformis*	LTF
Juglandaceae	*Carya*	*cordiformis*	DTF
Salicaceae	*Casearia*	*arborea*	LTF
Ericaceae	*Cavendishia*	*bracteata*	UMF
Urticaceae	*Cecropia*	*peltata*	LTF
Meliaceae	*Cedrela*	*montana*	UMF

Family	Genus	Species	Site
Meliaceae	*Cedrela*	*odorata*	LTF
Cannabaceae	*Celtis*	*occidentalis*	DTF
Ochnaceae	*Cespedesia*	*spathulata*	LTF
Verbenaceae	*Citharexylum*	*dryanderae*	UMF
Leguminosae	*Clathrotropis*	*brachypetala*	LTF
Clusiaceae	*Clusia*	*multiflora*	UMF
Boraginaceae	*Cordia*	*alliodora*	LTF
Boraginaceae	*Cordia*	*cylindrostachya*	UMF
Boraginaceae	*Cordia spp.*		UMF
Euphorbiaceae	*Croton*	*killipianus*	LTF
Winteraceae	*Drimys*	*granadensis*	UMF
Annonaceae	*Duguetia*	*antioquensis*	LTF
Fagaceae	*Fagus*	*grandifolia*	DTF
Oleaceae	*Fraxinus*	*americana*	DTF
Loranthaceae	*Gaiadendron*	*punctatum*	UMF
Malvaceae	*Goethalsia*	*meiantha*	LTF
Goupiaceae	*Goupia*	*glabra*	LTF
Phyllanthaceae	*Hieronyma*	*alchorneoides*	LTF
Leguminosae	*Hymenaea*	*courbaril*	LTF
Lamiaceae	*Hyptidendron*	*arboreum*	LTF
Aquifoliaceae	*Ilex*	*nervosa*	UMF
Myristicaceae	*Iryanthera*	*megistocarpa*	LTF
Juglandaceae	*Juglans*	*cinerea*	DTF
Juglandaceae	*Juglans*	*neotropica*	UMF
Rubiaceae	*Ladembergia spp.*		LTF
Lecythidaceae	*Lecythis*	*ampla*	LTF
Melastomataceae	*Miconia*	*biappendiculata*	UMF
Myricaceae	*Morella*	*parvifolia*	UMF
Muntingiaceae	*Muntingia*	*calabura*	LTF
Myrtaceae	*Myrcianthes*	*leucoxyla*	UMF
Primulaceae	*Myrsine*	*coriacea*	UMF

Family	Genus	Species	Site
Primulaceae	*Myrsine*	*coriacea*	UMF
Anacardiaceae	*Ochoterenaea*	*colombiana*	LTF
Malvaceae	*Ochroma*	*pyramidale*	LTF
Araliaceae	*Oreopanax*	*bogotensis*	UMF
Betulaceae	*Ostrya*	*virginiana*	DTF
Piperaceae	*Piper*	*bogotense*	UMF
Compositae	*Piptocoma*	*discolor*	LTF
Salicaceae	*Populus*	*grandidentata*	DTF
Salicaceae	*Populus*	*tremuloides*	DTF
Burseraceae	*Protium*	*aracouchini*	LTF
Rosaceae	*Prunus*	*buxifolia*	UMF
Rosaceae	*Prunus*	*pensylvanica*	DTF
Rosaceae	*Prunus*	*serotina*	DTF
Annonaceae	*Pseudoxandra*	*sclerocarpa*	LTF
Fagaceae	*Quercus*	*rubra*	DTF
Rhamnaceae	*Frangula*	*goudotiana*	UMF
Solanaceae	*Solanum*	*humboldtianum*	UMF
Bignoniaceae	*Handroanthus*	*guayacan*	LTF
Anacardiaceae	*Tapirira*	*guianensis*	LTF
Malvaceae	*Tilia*	*americana*	DTF
Malvaceae	*Tilia*	*cordata*	DTF
Cannabaceae	*Trema*	*micrantha*	UMF
Ulmaceae	*Ulmus*	*americana*	DTF
Compositae	*Verbesina*	*crassiramea*	UMF
Adoxaceae	*Viburnum*	*lasiophyllum*	UMF
Hypericaceae	*Vismia*	*macrophylla*	LTF
Rutaceae	*Zanthoxylum spp.*		UMF

2.7.2 Annex B

Table 2.6B Contribution of traits and carbohydrate concentrations to the first two principal components in the multiple factor analysis.

	MFA LTF		MFA UMF		MFA DTF	
	Dim1 (20.4*)	Dim2 (16.7%)	Dim1 (29.5%)	Dim2 (16.8%)	Dim1 (21.6%)	Dim2 (14.9%)
LS	1.79	0.40	0.10	4.21	0.33	8.62
LT	0.41	3.26	9.69	0.58	0.00	22.84
LDMC	7.77	1.33	2.63	11.53	10.30	4.36
SLA	4.54	3.77	9.38	5.43	3.05	16.18
Amax_area	9.18	0.00	1.93	4.69	1.96	7.59
Amax_mass	12.03	1.25	7.72	8.17	5.35	0.77
N	4.94	1.36	8.55	6.41	10.56	0.59
K	4.17	1.07	5.36	8.26	0.72	0.09
Mg	0.03	2.65	0.25	0.00	4.82	0.16
SD	6.56	2.37	1.45	5.12	6.45	5.95
RD	4.26	0.94	0.73	2.33	7.29	3.22
H	6.33	0.00	0.62	0.33	1.76	0.99
NSC_R	32.72	2.12	10.33	3.85	8.45	1.85
NSC_S	0.71	45.86	13.37	23.37	1.78	13.74
NSC_B	2.79	33.33	18.02	6.18	12.29	12.00
NSC_L	1.76	0.29	9.85	9.54	24.89	1.05

2.7.3 Annex C

Table 2.1C Standardized major axis (SMA) relationships between carbohydrate concentrations and functional traits.

Relationships are presented by biome with their tests of differences in slope. LTF: lowland tropical rainforest, UMF: upper montane forest, and DTF: deciduous temperate forest.

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
LS	sugar_R	153	0.00	0.45	47	0.02	0.37	47	0.00	0.72	59	0.00	0.85	0.019	0.006	0.561	0.042
LS	starch_R	145	0.00	0.98	42	0.03	0.28	43	0.00	0.98	58	0.04	0.16	0.001	0.000	0.507	0.008
LS	NSC_R	154	0.00	0.82	47	0.03	0.22	47	0.01	0.48	58	0.03	0.16	0.053	0.018	0.139	0.398
LS	sugar_S	123	0.01	0.34	28	0.01	0.66	36	0.07	0.11	49	0.03	0.26	0.000	0.000	0.000	0.915
LS	starch_S	102	0.02	0.12	23	0.01	0.66	29	0.26	**0.00**	43	0.06	0.10	0.000	0.000	0.006	0.105
LS	NSC_S	129	0.01	0.18	28	0.03	0.37	37	0.10	0.06	54	0.00	0.70	0.123	0.041	0.467	0.182
LS	sugar_B	138	0.02	0.10	20	0.00	0.91	46	0.16	**0.01**	58	0.03	0.19	0.002	0.055	0.001	0.632
LS	starch_B	134	0.01	0.41	21	0.04	0.41	42	0.00	0.69	58	0.01	0.42	0.092	0.192	0.211	0.031
LS	NSC_B	139	0.01	0.30	21	0.06	0.28	46	0.01	0.45	58	0.00	0.96	0.037	0.017	0.922	0.017
LS	sugar_L	148	0.07	**0.00**	36	0.02	0.39	43	0.12	**0.03**	55	0.01	0.41	0.005	0.195	0.001	0.091
LS	starch_L	148	0.00	0.96	35	0.00	0.88	43	0.03	0.28	55	0.03	0.24	0.003	0.002	0.013	0.457
LS	NSC_L	153	0.02	0.11	37	0.00	0.87	46	0.01	0.64	55	0.00	0.78	0.044	0.014	0.535	0.069

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
LT	sugar_R	153	0.01	0.23	47	0.01	0.60	47	0.00	0.84	59	0.17	**0.00**	0.438	0.199	0.587	0.500
LT	starch_R	145	0.09	**0.00**	42	0.00	0.66	43	0.26	**0.00**	58	0.00	0.91	0.834	0.935	0.569	0.660
LT	NSC_R	154	0.08	**0.00**	47	0.02	0.38	47	0.28	**0.00**	58	0.00	0.74	0.123	0.153	0.552	0.044
LT	sugar_S	123	0.07	**0.00**	28	0.09	0.11	36	0.13	**0.03**	49	0.04	0.19	0.006	0.170	0.001	0.137
LT	starch_S	102	0.01	0.31	23	0.00	0.97	29	0.34	**0.00**	43	0.02	0.37	0.881	0.619	0.788	0.783
LT	NSC_S	129	0.01	0.22	28	0.10	0.10	37	0.43	**0.00**	54	0.01	0.50	0.115	0.088	0.070	0.804
LT	sugar_B	138	0.03	**0.03**	20	0.06	0.28	46	0.06	0.11	58	0.02	0.30	0.003	0.143	0.013	0.002
LT	starch_B	134	0.01	0.22	21	0.00	0.80	42	0.16	**0.01**	58	0.08	**0.03**	0.035	0.031	0.042	0.511
LT	NSC_B	139	0.00	0.43	21	0.06	0.27	46	0.14	**0.01**	58	0.09	**0.02**	0.446	0.270	0.337	0.696
LT	sugar_L	148	0.26	**0.00**	36	0.04	0.23	43	0.01	0.58	55	0.00	0.88	0.000	0.000	0.000	0.298
LT	starch_L	148	0.03	**0.03**	35	0.08	0.10	43	0.00	0.81	55	0.00	0.81	0.506	0.730	0.384	0.265
LT	NSC_L	153	0.27	**0.00**	37	0.17	**0.01**	46	0.06	0.10	55	0.01	0.53	0.479	0.228	0.505	0.586
LDMC	sugar_R	153	0.03	**0.04**	47	0.00	0.78	47	0.09	**0.04**	59	0.00	0.75	0.792	0.549	0.573	0.962
LDMC	starch_R	145	0.01	0.28	42	0.03	0.27	43	0.01	0.65	58	0.03	0.21	0.876	0.687	0.903	0.627
LDMC	NSC_R	154	0.02	0.13	47	0.08	0.06	47	0.01	0.56	58	0.02	0.25	0.424	0.349	0.691	0.206
LDMC	sugar_S	123	0.03	0.05	28	0.02	0.50	36	0.02	0.36	49	0.03	0.24	0.001	0.034	0.000	0.195
LDMC	starch_S	102	0.01	0.22	23	0.01	0.63	29	0.00	0.95	43	0.01	0.50	0.547	0.327	0.413	0.836
LDMC	NSC_S	129	0.03	**0.04**	28	0.01	0.60	37	0.00	0.82	54	0.00	0.70	0.572	0.391	0.374	0.965
LDMC	sugar_B	138	0.02	0.10	20	0.00	0.96	46	0.01	0.59	58	0.07	0.05	0.042	0.956	0.017	0.085

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
LDMC	starch_B	134	0.00	0.68	21	0.03	0.45	42	0.02	0.41	58	0.00	0.70	0.199	0.461	0.073	0.526
LDMC	NSC_B	139	0.00	0.41	21	0.07	0.23	46	0.00	0.78	58	0.02	0.27	0.450	0.711	0.310	0.267
LDMC	sugar_L	148	0.06	**0.00**	36	0.01	0.65	43	0.02	0.34	55	0.02	0.34	0.000	0.000	0.000	0.479
LDMC	starch_L	148	0.00	0.50	35	0.04	0.26	43	0.13	**0.02**	55	0.28	**0.00**	0.407	0.857	0.191	0.358
LDMC	NSC_L	153	0.02	0.10	37	0.03	0.27	46	0.01	0.53	55	0.30	**0.00**	0.731	0.455	0.614	0.803
SLA	sugar_R	153	0.00	0.91	47	0.00	0.76	47	0.04	0.19	59	0.13	**0.01**	0.028	0.008	0.207	0.183
SLA	starch_R	145	0.04	**0.02**	42	0.05	0.15	43	0.11	**0.03**	58	0.00	0.91	0.325	0.149	0.319	0.650
SLA	NSC_R	154	0.05	**0.01**	47	0.03	0.28	47	0.13	**0.01**	58	0.00	0.83	0.007	0.004	0.793	0.009
SLA	sugar_S	123	0.04	**0.02**	28	0.06	0.19	36	0.01	0.51	49	0.00	0.81	0.034	0.782	0.023	0.025
SLA	starch_S	102	0.00	0.97	23	0.14	0.08	29	0.27	**0.00**	43	0.02	0.32	0.606	0.317	0.696	0.521
SLA	NSC_S	129	0.00	0.55	28	0.19	**0.02**	37	0.25	**0.00**	54	0.02	0.34	0.002	0.001	0.016	0.201
SLA	sugar_B	138	0.02	0.08	20	0.03	0.47	46	0.02	0.32	58	0.01	0.55	0.006	0.055	0.066	0.002
SLA	starch_B	134	0.01	0.25	21	0.04	0.41	42	0.03	0.24	58	0.05	0.08	0.007	0.007	0.017	0.393
SLA	NSC_B	139	0.01	0.32	21	0.08	0.22	46	0.06	0.10	58	0.05	0.09	0.139	0.096	0.116	0.643
SLA	sugar_L	148	0.20	**0.00**	36	0.00	0.68	43	0.00	0.97	55	0.10	**0.02**	0.000	0.000	0.000	0.159
SLA	starch_L	148	0.06	**0.00**	35	0.17	**0.01**	43	0.03	0.26	55	0.04	0.17	0.198	0.170	0.633	0.083
SLA	NSC_L	153	0.24	**0.00**	37	0.11	**0.04**	46	0.05	0.13	55	0.12	**0.01**	0.071	0.023	0.192	0.327
Amax_area	sugar_R	153	0.02	0.08	47	0.01	0.57	47	0.06	0.11	59	0.04	0.11	0.292	0.948	0.150	0.198
Amax_area	starch_R	145	0.00	0.82	42	0.12	**0.02**	43	0.04	0.20	58	0.00	0.72	0.190	0.345	0.321	0.068

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
Amax_area	NSC_R	154	0.01	0.28	47	0.21	**0.00**	47	0.08	**0.05**	58	0.00	0.70	0.903	0.726	0.677	0.937
Amax_area	sugar_S	123	0.00	0.67	28	0.01	0.59	36	0.00	0.96	49	0.01	0.43	0.002	0.006	0.002	0.960
Amax_area	starch_S	102	0.00	0.51	23	0.08	0.18	29	0.02	0.49	43	0.00	0.88	0.105	0.034	0.427	0.200
Amax_area	NSC_S	129	0.01	0.22	28	0.21	**0.01**	37	0.01	0.58	54	0.01	0.49	0.222	0.854	0.097	0.187
Amax_area	sugar_B	138	0.01	0.20	20	0.02	0.52	46	0.03	0.24	58	0.00	0.97	0.225	0.857	0.118	0.195
Amax_area	starch_B	134	0.01	0.20	21	0.12	0.12	42	0.01	0.65	58	0.01	0.59	0.017	0.473	0.004	0.127
Amax_area	NSC_B	139	0.00	0.92	21	0.05	0.34	46	0.04	0.21	58	0.00	0.61	0.105	0.681	0.068	0.083
Amax_area	sugar_L	148	0.01	0.18	36	0.00	0.77	43	0.01	0.47	55	0.02	0.26	0.000	0.000	0.000	0.424
Amax_area	starch_L	148	0.04	**0.02**	35	0.01	0.48	43	0.02	0.34	55	0.14	**0.01**	0.637	0.346	0.648	0.631
Amax_area	NSC_L	153	0.06	**0.00**	37	0.01	0.52	46	0.02	0.36	55	0.06	0.08	0.306	0.997	0.161	0.212
Amax_mass	sugar_R	153	0.03	**0.03**	47	0.01	0.52	47	0.00	1.00	59	0.06	0.06	0.341	0.452	0.145	0.506
Amax_mass	starch_R	145	0.03	**0.03**	42	0.17	**0.01**	43	0.11	**0.03**	58	0.07	0.05	0.127	0.076	0.096	0.930
Amax_mass	NSC_R	154	0.04	**0.01**	47	0.22	**0.00**	47	0.15	**0.01**	58	0.10	**0.01**	0.029	0.638	0.011	0.061
Amax_mass	sugar_S	123	0.05	**0.01**	28	0.00	0.87	36	0.00	0.70	49	0.06	0.09	0.000	0.000	0.000	0.195
Amax_mass	starch_S	102	0.04	**0.03**	23	0.00	0.77	29	0.07	0.17	43	0.01	0.52	0.020	0.013	0.037	0.569
Amax_mass	NSC_S	129	0.07	**0.00**	28	0.04	0.33	37	0.08	0.10	54	0.00	0.75	0.894	0.746	0.658	0.944
Amax_mass	sugar_B	138	0.02	0.10	20	0.00	0.88	46	0.04	0.19	58	0.08	**0.03**	0.000	0.508	0.000	0.000
Amax_mass	starch_B	134	0.01	0.30	21	0.06	0.28	42	0.03	0.29	58	0.03	0.21	0.496	0.251	0.921	0.307
Amax_mass	NSC_B	139	0.01	0.16	21	0.00	0.87	46	0.07	0.08	58	0.06	0.06	0.522	0.972	0.286	0.427

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
Amax_mass	sugar_L	148	0.22	**0.00**	36	0.01	0.63	43	0.00	0.68	55	0.09	**0.02**	0.010	0.004	0.045	0.349
Amax_mass	starch_L	148	0.04	**0.01**	35	0.02	0.41	43	0.00	0.84	55	0.04	0.17	0.007	0.092	0.002	0.237
Amax_mass	NSC_L	153	0.21	**0.00**	37	0.01	0.52	46	0.01	0.63	55	0.07	**0.04**	0.321	0.373	0.140	0.642
N	sugar_R	153	0.00	0.88	47	0.00	0.88	47	0.02	0.37	59	0.10	**0.02**	0.126	0.812	0.082	0.067
N	starch_R	145	0.02	0.11	42	0.13	**0.02**	43	0.13	**0.02**	58	0.00	0.80	0.112	0.384	0.037	0.245
N	NSC_R	154	0.03	**0.02**	47	0.05	0.12	47	0.16	**0.01**	58	0.00	0.72	0.003	0.559	0.007	0.002
N	sugar_S	123	0.00	0.99	28	0.01	0.56	36	0.01	0.66	49	0.17	**0.00**	0.000	0.029	0.000	0.008
N	starch_S	102	0.02	0.16	23	0.00	0.76	29	0.09	0.11	43	0.00	0.84	0.031	0.163	0.009	0.370
N	NSC_S	129	0.02	0.15	28	0.03	0.38	37	0.08	0.08	54	0.02	0.29	0.319	0.288	0.543	0.132
N	sugar_B	138	0.00	0.50	20	0.05	0.33	46	0.00	0.68	58	0.03	0.18	0.000	0.427	0.000	0.000
N	starch_B	134	0.04	**0.03**	21	0.02	0.50	42	0.05	0.15	58	0.03	0.19	0.220	0.160	0.597	0.084
N	NSC_B	139	0.04	**0.02**	21	0.01	0.75	46	0.06	0.11	58	0.05	0.09	0.303	0.758	0.183	0.211
N	sugar_L	148	0.02	0.12	36	0.04	0.25	43	0.00	0.98	55	0.03	0.24	0.001	0.000	0.101	0.045
N	starch_L	148	0.01	0.25	35	0.06	0.17	43	0.01	0.50	55	0.25	**0.00**	0.001	0.298	0.000	0.026
N	NSC_L	153	0.03	**0.02**	37	0.00	0.78	46	0.02	0.40	55	0.26	**0.00**	0.122	0.947	0.052	0.114
K	sugar_R	153	0.00	0.50	47	0.01	0.42	47	0.06	0.09	59	0.01	0.47	0.035	0.363	0.010	0.116
K	starch_R	145	0.00	0.91	42	0.12	**0.02**	43	0.06	0.12	58	0.11	**0.01**	0.050	0.820	0.033	0.029
K	NSC_R	154	0.00	0.48	47	0.03	0.28	47	0.07	0.08	58	0.12	**0.01**	0.203	0.203	0.094	0.720
K	sugar_S	123	0.02	0.12	28	0.02	0.51	36	0.00	0.87	49	0.07	0.06	0.142	0.726	0.053	0.183

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
K	starch_S	102	0.00	0.90	23	0.00	0.93	29	0.00	0.86	43	0.06	0.12	0.429	0.964	0.229	0.293
K	NSC_S	129	0.01	0.33	28	0.00	0.81	37	0.02	0.37	54	0.00	0.84	0.004	0.009	0.004	0.966
K	sugar_B	138	0.03	**0.03**	20	0.00	0.96	46	0.00	0.90	58	0.21	**0.00**	0.734	0.621	0.650	0.439
K	starch_B	134	0.01	0.22	21	0.07	0.24	42	0.00	0.99	58	0.03	0.19	0.001	0.209	0.000	0.115
K	NSC_B	139	0.06	**0.00**	21	0.10	0.15	46	0.01	0.48	58	0.10	**0.02**	0.008	0.886	0.003	0.039
K	sugar_L	148	0.05	**0.01**	36	0.10	0.07	43	0.06	0.13	55	0.05	0.10	0.000	0.000	0.000	0.198
K	starch_L	148	0.01	0.35	35	0.00	0.78	43	0.11	**0.03**	55	0.00	0.67	0.598	0.790	0.438	0.346
K	NSC_L	153	0.01	0.18	37	0.02	0.43	46	0.00	0.74	55	0.02	0.30	0.039	0.543	0.012	0.094
Mg	sugar_R	153	0.01	0.18	47	0.19	**0.00**	47	0.09	**0.04**	59	0.02	0.24	0.000	0.876	0.000	0.000
Mg	starch_R	145	0.00	0.61	42	0.00	0.89	43	0.02	0.39	58	0.06	0.05	0.000	0.324	0.000	0.000
Mg	NSC_R	154	0.00	0.61	47	0.00	0.88	47	0.02	0.36	58	0.06	0.05	0.000	0.694	0.000	0.002
Mg	sugar_S	123	0.04	**0.03**	28	0.00	0.81	36	0.01	0.59	49	0.05	0.14	0.128	0.099	0.087	0.938
Mg	starch_S	102	0.04	0.06	23	0.03	0.44	29	0.00	0.88	43	0.00	0.66	0.800	0.870	0.587	0.538
Mg	NSC_S	129	0.12	**0.00**	28	0.26	**0.01**	37	0.01	0.67	54	0.01	0.43	0.006	0.114	0.002	0.145
Mg	sugar_B	138	0.04	**0.01**	20	0.03	0.45	46	0.07	0.08	58	0.09	**0.02**	0.179	0.361	0.069	0.690
Mg	starch_B	134	0.00	0.79	21	0.03	0.42	42	0.00	0.99	58	0.02	0.33	0.000	0.111	0.000	0.005
Mg	NSC_B	139	0.01	0.39	21	0.07	0.23	46	0.00	0.88	58	0.05	0.08	0.000	0.609	0.000	0.001
Mg	sugar_L	148	0.08	**0.00**	36	0.00	0.91	43	0.30	**0.00**	55	0.11	**0.02**	0.000	0.000	0.000	0.000
Mg	starch_L	148	0.00	0.56	35	0.03	0.31	43	0.08	0.06	55	0.25	**0.00**	0.001	0.207	0.004	0.000

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
Mg	NSC_L	153	0.04	**0.02**	37	0.07	0.12	46	0.04	0.20	55	0.32	**0.00**	0.000	0.803	0.000	0.000
SD	sugar_R	153	0.02	0.10	47	0.04	0.19	47	0.00	0.87	59	0.05	0.08	0.002	0.757	0.003	0.002
SD	starch_R	145	0.01	0.19	42	0.07	0.09	43	0.00	0.76	58	0.01	0.53	0.001	0.184	0.008	0.000
SD	NSC_R	154	0.01	0.25	47	0.08	0.05	47	0.03	0.23	58	0.00	0.71	0.074	0.990	0.042	0.050
SD	sugar_S	123	0.00	0.74	28	0.00	0.88	36	0.03	0.29	49	0.08	**0.05**	0.002	0.001	0.017	0.262
SD	starch_S	102	0.00	0.52	23	0.01	0.73	29	0.01	0.64	43	0.03	0.23	0.007	0.002	0.815	0.009
SD	NSC_S	129	0.00	0.85	28	0.00	0.87	37	0.00	0.88	54	0.00	0.80	0.020	0.732	0.014	0.017
SD	sugar_B	138	0.01	0.38	20	0.05	0.34	46	0.00	0.68	58	0.01	0.47	0.853	0.591	0.992	0.609
SD	starch_B	134	0.00	0.58	21	0.02	0.58	42	0.00	0.71	58	0.08	**0.03**	0.000	0.397	0.000	0.022
SD	NSC_B	139	0.01	0.19	21	0.01	0.71	46	0.01	0.44	58	0.07	**0.04**	0.001	0.797	0.001	0.008
SD	sugar_L	148	0.00	0.41	36	0.00	0.83	43	0.01	0.56	55	0.00	0.63	0.000	0.001	0.000	0.012
SD	starch_L	148	0.02	0.09	35	0.00	0.99	43	0.00	0.72	55	0.37	**0.00**	0.021	0.130	0.074	0.006
SD	NSC_L	153	0.01	0.25	37	0.01	0.50	46	0.02	0.33	55	0.27	**0.00**	0.001	0.606	0.001	0.001
RD	sugar_R	153	0.04	**0.01**	47	0.10	**0.03**	47	0.00	0.82	59	0.13	**0.01**	0.002	0.072	0.000	0.083
RD	starch_R	145	0.01	0.28	42	0.07	0.09	43	0.00	0.81	58	0.00	0.68	0.005	0.471	0.002	0.021
RD	NSC_R	154	0.00	0.51	47	0.07	0.06	47	0.01	0.59	58	0.02	0.35	0.020	0.035	0.010	0.610
RD	sugar_S	123	0.01	0.22	28	0.01	0.59	36	0.01	0.54	49	0.04	0.18	0.310	0.310	0.149	0.770
RD	starch_S	102	0.00	0.49	23	0.04	0.37	29	0.00	0.76	43	0.00	0.69	0.287	0.130	0.897	0.199
RD	NSC_S	129	0.00	0.92	28	0.02	0.46	37	0.00	0.97	54	0.01	0.41	0.004	0.054	0.001	0.320

Y-variable	X-variable	Model all trees			Model trees LTF			Model trees UMF			Model trees DTF			Same slope test	Pairwise slope comparison		
		n	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value	*n*	r^2	Model *p*-value		DTF vs LTF	DTF vs UMF	LTF vs UMF
RD	sugar_B	138	0.01	0.22	20	0.01	0.64	46	0.02	0.33	58	0.03	0.22	0.285	0.114	0.546	0.268
RD	starch_B	134	0.01	0.29	21	0.01	0.68	42	0.00	0.70	58	0.06	0.05	0.000	0.026	0.000	0.215
RD	NSC_B	139	0.01	0.23	21	0.05	0.33	46	0.00	0.70	58	0.08	**0.04**	0.000	0.242	0.000	0.071
RD	sugar_L	148	0.00	0.59	36	0.01	0.54	43	0.00	0.86	55	0.25	**0.00**	0.000	0.000	0.000	0.260
RD	starch_L	148	0.00	0.50	35	0.03	0.35	43	0.02	0.43	55	0.10	**0.02**	0.189	0.556	0.069	0.295
RD	NSC_L	153	0.00	0.64	37	0.01	0.67	46	0.01	0.46	55	0.26	**0.00**	0.001	0.109	0.000	0.090
H	sugar_R	153	0.01	0.35	47	0.02	0.36	47	0.00	0.97	59	0.00	0.73	0.000	0.000	0.029	0.102
H	starch_R	145	0.08	**0.00**	42	0.15	**0.01**	43	0.01	0.45	58	0.03	0.21	0.006	0.002	0.089	0.184
H	NSC_R	154	0.08	**0.00**	47	0.28	**0.00**	47	0.00	0.88	58	0.03	0.16	0.000	0.000	0.213	0.003
H	sugar_S	123	0.04	**0.02**	28	0.04	0.31	36	0.01	0.66	49	0.00	0.91	0.006	0.991	0.003	0.010
H	starch_S	102	0.02	0.16	23	0.01	0.66	29	0.01	0.60	43	0.00	0.79	0.517	0.434	0.619	0.253
H	NSC_S	129	0.04	**0.03**	28	0.02	0.52	37	0.00	0.99	54	0.00	0.96	0.005	0.002	0.054	0.192
H	sugar_B	138	0.02	0.08	20	0.14	0.11	46	0.00	0.95	58	0.00	0.75	0.014	0.016	0.422	0.004
H	starch_B	134	0.00	0.62	21	0.06	0.27	42	0.02	0.37	58	0.00	0.71	0.001	0.003	0.001	0.662
H	NSC_B	139	0.00	0.72	21	0.01	0.68	46	0.03	0.29	58	0.00	0.82	0.020	0.058	0.010	0.985
H	sugar_L	148	0.10	**0.00**	36	0.10	0.06	43	0.04	0.20	55	0.05	0.10	0.000	0.000	0.000	0.016
H	starch_L	148	0.00	0.46	35	0.01	0.63	43	0.04	0.22	55	0.06	0.07	0.005	0.002	0.771	0.007
H	NSC_L	153	0.04	**0.01**	37	0.01	0.63	46	0.01	0.52	55	0.02	0.37	0.001	0.000	0.033	0.069

2.8 REFERENCES

Atkinson, R.R.L., Burrell, M.M., Osborne, C.P., Rose, K.E. & Rees, M. (2012) A non-targeted metabolomics approach to quantifying differences in root storage between fast- and slow-growing plants. *New Phytologist,* **196,** 200–211.

Atkinson, R.R.L., Burrell, M.M., Rose, K.E., Osborne, C.P. & Rees, M. (2014) The dynamics of recovery and growth: how defoliation affects stored resources. *Proceedings of the Royal Society B,* **281,** 20133355.

Baraloto, C., Paine, C.E.T., Poorter, L., Beauchene, J., Bonal, D., Domenach, A.-M., Hérault, B., patiño, S., Roggy, J.-C. & Chave, J. (2010) Decoupled leaf and stem economics in rain forest trees. *Ecology Letters,* **13,** 1338-1347.

Campbell, C., Atkinson, L., Zaragoza-Castells, J., Lundmark, M., Atkin, O. & Hurry, V. (2007) Acclimation of photosynthesis and respiration is asynchronous in response to changes in temperature regardless of plant functional group. *New Phytologist,* **176,** 375–389.

Canham, C.D., Kobe, R.K., Latty, E.F. & Chazdon, R.L. (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. *Oecologia,* **121,** 1-11.

Carbone, M.S., Czimczik, C.I., Keenan, T.F., Murakami, P.F., Pederson, N., Schaberg, P.G., Xu, X. & Richardson, A.D. (2013) Age, allocation and availability of nonstructural carbon in mature red maple trees. *New Phytologist,* **200,** 1145–1155.

Clark, D.B. & Clark, D.A. (1991) The impact of physical damage on canopy tree regeneration in tropical rain forest *Journal of Ecology,* **79,** 447-457.

Clarke, P.J., Lawes, M.J., Midgley, J.J., Lamont, B.B., Ojeda, F., Burrows, G.E., Enright, N.J. & Knox, K.J.E. (2013) Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytologist,* **197,** 19–35.

Coley, P.D. & Barone, J.A. (1996) Herbivory and Plant Defenses in Tropical Forests. *Annual Review of Ecology and Systematics,* **27,** 305-335.

Coley, P.D., Bryant, J.P. & Chapin, F.S. (1985) Resource availability and plant antiherbivore defense. *Science,* **230,** 895-899.

Chapin, F.S., Schulze, E.D. & Mooney, H.A. (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics,* **21,** 423-447.

Chave, J., Coomes, D., Jansen, S., Lewis, S.L., Swenson, N.G. & Zanne, A.E. (2009) Towards a worldwide wood economics spectrum. *Ecology Letters,* **12,** 351–366.

Diaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C., Jalili, A., Montserrat-Martí, G., Grime, J.P., Zarrinkamar, F., Asri, Y., Band, S.R., Basconcelo, S., Castro-Díez, P., Funes, G., Hamzehee, B., Khoshnevi, M.,

Pérez-Harguindeguy, N., Pérez-Rontomé, M.C., Shirvany, F.A., Vendramini, F., Yazdani, S., Abbas-Azimi, R., Bogaard, A., Boustani, S., Charles, M., Dehghan, M., de_Torres-Espuny, L., Falczuk, V., Guerrero-Campo, J., Hynd, A., Jones, G., Kowsary, E., Kazemi-Saeed, F., Maestro-Martínez, M., Romo-Díez, A., Shaw, S., Siavash, B., Villar-Salvador, P. & Zak, M.R. (2004) The plant traits that drive ecosystems: evidence from three continents. *Journal of Vegetation Science,* **15,** 295–304.

Díaz, S., Kattge, J., Cornelissen, J.H.C., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C., Colin Prentice, I., Garnier, E., Bönisch, G., Westoby, M., Poorter, H., Reich, P.B., Moles, A.T., Dickie, J., Gillison, A.N., Zanne, A.E., Chave, J., Joseph Wright, S., Sheremet'ev, S.N., Jactel, H., Baraloto, C., Cerabolini, B., Pierce, S., Shipley, B., Kirkup, D., Casanoves, F., Joswig, J.S., Günther, A., Falczuk, V., Rüger, N., Mahecha, M.D. & Gorné, L.D. (2016) The global spectrum of plant form and function. *Nature,* **529,** 167-171.

Dietze, M.C., Sala, A., Carbone, M.S., Czimczik, C.I., Mantooth, J.A., Richardson, A.D. & Vargas, R. (2013) Nonstructural Carbon in Woody Plants. *Annual Review of Plant Biology,* **65,** 2.1–2.21.

Donovan, L.A., Maherali, H., Caruso, C.M., Huber, H. & de_Kroon, H. (2011) The evolution of the worldwide leaf economics spectrum. *Trends in ecology and evolution,* **26,** 88–95.

Escofier, B. & Pages, J. (1994) Multiple factor analysis (AFMULT package). *Computational Statistics and Data Analysis,* **18,** 121–140.

Fajardo, A. & Piper, F.I. (2014) An experimental approach to explain the Southern Andes elevational treeline. *American Journal of Botany,* **101,** 788–795.

Gaucher, C., Gougeon, S., Mauffette, Y. & Messier, C. (2005) Seasonal variation in biomass and carbohydrate partitioning of understory sugar maple (Acer saccharum) and yellow birch (Betula alleghaniensis) seedlings. *Tree Physiology,* **25,** 93–100.

Genet, H., Bréda, N. & Dufrêne, E. (2009) Age-related variation in carbon allocation at tree and stand scales in beech (Fagus sylvatica L.) and sessile oak (Quercus petraea (Matt.) Liebl.) using a chronosequence approach. *Tree Physiology,* **30,** 177–192.

Gough, C.M., Flower, C.E., Vogel, C.S., Dragoni, D. & Curtis, P.S. (2009) Whole-ecosystem labile carbon production in a north temperate deciduous forest. *Agricultural and forest meteorology,* **149,** 1531-1540.

Grime, J.P., Thompson, K., Hunt, R., Hodgson, J.G., Cornelissen, J.H.C., Rorison, I.H., Hendry, G.A.F., Ashenden, T.W., Askew, A.P., Band, S.R., Booth, R.E., Bossard, C.C., Campbell, B.D., Cooper, J.E.L., Davison, A.W., Gupta, P.L., Hall, W., Hand, D.W., Hannah, M.A., Hillier, S.H., Hodkinson, D.J., Jalili, A., Liu, Z., Mackey, J.M.L., Matthews, N., Mowforth, M.A., Neal, A.M.,

Reader, R.J., Reiling, K., Ross-Fraser, W., Spencer, R.E., Sutton, F., Tasker, D.E., Thorpe, P.C. & Whitehouse, J. (1997) Integrated screening validates primary axes of specialisation in plants. *Oikos,* **79,** 259–281.

Handa, T., Körner, C. & Hättenschwiler, S. (2005) A test of the treeline carbon limitation hypothesis by in situ CO2 enrichment and defoliation. *Ecology,* **86,** 1288-1300.

Hartmann, H. & Trumbore, S. (2016) Understanding the roles of nonstructural carbohydrates in forest trees – from what we can measure to what we want to know. *New Phytologist.*

Heberling, J.M. & Fridley, J.D. (2012) Biogeographic constraints on the world-wide leaf economics spectrum. *Global Ecology and Biogeography,* **21,** 1137–1146.

Heberling, J.M. & Fridley, J.D. (2013) Resource-use strategies of native and invasive plants in Eastern North American forests. *New Phytologist,* **200,** 523–533.

Hoch, G. (2015) Carbon Reserves as Indicators for Carbon Limitation in Trees. *Progress in Botany: Vol. 76* (eds U. Lüttge & W. Beyschlag), pp. 321-346. Springer International Publishing, Cham.

Hoch, G. & Körner, C. (2012) Global patterns of mobile carbon stores in trees at the high-elevation tree line. *Global Ecology and Biogeography,* **21,** 861-871.

Hoch, G., Popp, M. & Körner, C. (2002) Altitudinal increase of mobile carbon pools in Pinus cembra suggests sink limitation of growth at the Swiss treeline. *Oikos,* **98,** 361–374.

Hoch, G., Richter, A. & Körner, C. (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell and Environment,* **26,** 1067-1081.

Imaji, A. & Seiwa, K. (2010) Carbon allocation to defense, storage, and growth in seedlings of two temperate broad-leaved tree species. *Oecologia,* **162,** 273–281.

Kennard, R.W. & Stone, L.A. (1969) Computer Aided Design of Experiments. *Technometrics,* **11,** 137-148.

Kitajima, K. (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia,* **98,** 419-428.

Klein, T., Vitasse, Y. & Hoch, G. (2016) Coordination between growth, phenology and carbon storage in three coexisting deciduous tree species in a temperate forest. *Tree Physiology,* **00,** 1-9.

Kobe, R.K. (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos,* **80,** 226-233.

Körner, C. (2003) Carbon limitation in trees. *Journal of Ecology,* **91,** 4-17.

Kramer, P.J. & Kozlowski, T.T. (1979) *Physiology pf Woody Plants.* Academic Press, Inc, New York.

Kuhn, M. (2008) Building predictive models in R using the caret package. *Journal of Statistical Software,* **28,** 1-26.

Lacointe, A. (2000) Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. *Annals of Forest Science,* **57,** 521–533.

Lê, S., Josse, J. & Husson, F. (2008) FactoMineR: an R package for multivariate analysis. *Journal of Statistical Software,* **25,** 1–18.

Li, N., He, N., Yu, G., Wang, Q. & Sun, J. (2016) Leaf non-structural carbohydrates regulated by plant functional groups and climate: Evidences from a tropical to cold-temperate forest transect. *Ecological Indicators,* **62,** 22-31.

Lusk, C.H. & Piper, F.I. (2007) Seedling size influences relationships of shade tolerance with carbohydrate-storage patterns in a temperate rainforest. *Functional Ecology,* **21,** 78-86.

Marino, G., Aqil, M. & Shipley, B. (2010) The leaf economics spectrum and the prediction of photosynthetic light–response curves. *Functional Ecology,* **24,** 263–272.

McDowell, N.G., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yepez, E.A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist,* **178,** 719–739.

Messier, C., Posada, J., Aubin, I. & Beaudet, M. (2009) Functional Relationships Between Old-Growth Forest Canopies, Understorey Light and Vegetation Dynamics. *Old Growth Forests: Function, Fate and Value, Ecological Studies 207* (eds C. Wirth, G. Gleixner & M. Heimann), pp. 115 - 139. Springer-Verlag, Berlin.

Mitchell, P.J., O'Grady, A.P., Tissue, D.T., White, D.A., Ottenschlaeger, M.L. & Pinkard, E.A. (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytologist,* **197,** 862-872.

Morris, H., Plavcová, L., Cvecko, P., Fichtler, E., Gillingham, M.A.F., Martínez-Cabrera, H.I., McGlinn, D.J., Wheeler, E., Zheng, J., Ziemińska, K. & Jansen, S. (2016) A global analysis of parenchyma tissue fractions in secondary xylem of seed plants. *New Phytologist,* **209,** 1553–1565.

Myers, J.A. & Kitajima, K. (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology,* **95,** 383-395.

Niklas, K. (1992) *Plant biomechanics: an engineering approach to plant form and function.* University of Chicago Press., Chicago, USA.

O'Brien, M.J., Leuzinger, S., Philipson, C.D., Tay, J. & Hector, A. (2014) Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate Change,* **4,** 710-714.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C., Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas, J.G., de_Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G., Thompson, K., Morgan, H.D., ter_Steege, H., van_der_Heijden, M.G.A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M.V., Conti, G., Staver, A.C., Aquino, S. & Cornelissen, J.H.C. (2013) New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany,* **61,** 167–234.

Piper, F.I. (2015) Patterns of carbon storage in relation to shade tolerance in southern South American species. *American Journal of Botany,* **102,** 1442-1452.

Piper, F.I. & Fajardo, A. (2011) No evidence of carbon limitation with tree age and height in Nothofagus pumilio under mediterranean and temperate climate conditions. *Annals of Botany,* **108,** 907–917.

Piper, F.I., Viñegla, B., Linares, J.C., Camarero, J.J., Cavieres, L.A. & Fajardo, A. (2016) Mediterranean and temperate treelines are controlled by different environmental drivers. *Journal of Ecology.*

Poorter, H., Niinemets, Ü., Poorter, L., Wright, I.J. & Villar, R. (2009) Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist,* **182,** 565–588.

Poorter, L. & Bongers, F. (2006) Leaf traits are good predictors of plant performance across 53 rain forest species. *Ecology,* **87,** 1733–1743.

Poorter, L. & Kitajima, K. (2007) Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology,* **88,** 1000-1011.

Poorter, L., Kitajima, K., Mercado, P., Chubina, J., Melgar, I. & Prins, H.H.T. (2010) Resprouting as a persistence strategy of tropical forest trees: relations with carbohydrate storage and shade tolerance. *Ecology,* **91,** 2613–2627.

Popp, M., Lied, W., Meyer, A.J., Richter, A., Schiller, P. & Schwitte, H. (1996) Sample preservation for determination of organic compounds: microwave versus freeze-drying. *Journal of Experimental Botany,* **47,** 1469-1473.

Ramirez, J.A., Posada, J.M., Handa, I.T., Hoch, G., Vohland, M., Messier, C. & Reu, B. (2015) Near-infrared spectroscopy (NIRS) predicts non-structural carbohydrate concentrations in different tissue types of a broad range of tree species. *Methods in Ecology and Evolution,* **6,** 1018–1025.

Reich, P.B. (2014) The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. *Journal of Ecology,* **102,** 275–301.

Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C. & Bowman, W.D. (1999) Generality of leaf trait relationships: a test across six biomes. *Ecology,* **80,** 1955–1969.

Reich, P.B., Walters, M.B. & Ellsworth, D.S. (1997) From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America,* **94,** 13730-13734.

Reich, P.B., Walters, M.B., Tjoelker, M.G., Vanderklein, D.W. & Bushena, C. (1998) Photosynthesis and respiration rates depend on leaf and root morphology and nitrogen concentration in nine boreal tree species differing in relative growth rate. *Functional Ecology,* **12,** 395–405.

Richardson, A.D., Carbone, M.S., Keenan, T.F., Czimczik, C.I., Hollinger, D.Y., Murakami, P., Schaberg, P.G. & Xu, X. (2013) Seasonal dynamics and age of stemwood nonstructural carbohydrates in temperate forest trees. *New Phytologist,* **197,** 850–861.

Ryan, M.G., Binkley, D. & Fownes, J.H. (1997) Age-related decline in forest productivity: pattern and process. *Advances in Ecological Research,* **27,** 213–262.

Sala, A. & Hoch, G. (2009) Height-related growth declines in ponderosa pine are not due to carbon limitation. *Plant, Cell & Environment,* **32,** 22–30.

Sala, A., Woodruff, D.R. & Meinzer, F. (2012) Carbon dynamics in trees: feast or famine? *Tree Physiology,* **32,** 764–775.

Schädel, C., Blöchl, A., Richter, A. & Hoch, G. (2009) Short-term dynamics of nonstructural carbohydrates and hemicelluloses in young branches of temperate forest trees during bud break. *Tree Physiology,* **29,** 901-911.

Tjoelker, M.G., Reich, P.B. & Oleksyn, J. (1999) Changes in leaf nitrogen and carbohydrates underlie temperature and CO2 acclimation of dark respiration in five boreal tree species. *Plant, Cell & Environment,* **22,** 767–778.

Tremblay, A. (2012) LMERConvenienceFunctions: a suite of functions to back-fit fixed effects and forward-fit random effects, as well as other miscellaneous functions.

Upmeyer, D.J. & Koller, H.R. (1973) Diurnal Trends in Net Photosynthetic Rate and Carbohydrate Levels of Soybean Leaves. *Plant Physiology,* **51,** 871-874.

Vargas, R., Trumbore, S.E. & Allen, M.F. (2009) Evidence of old carbon used to grow new fine roots in a tropical forest. *New Phytologist,* **182,** 710-718.

Warton, D.I., Duursma, R.A., Falster, D.S. & Taskinen, S. (2012) SMATR 3– an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution,* **3,** 257–259.

Warton, D.I., Wright, I.J., Falster, D.S. & Westoby, M. (2006) Bivariate line-fitting methods for allometry. *Biological Reviews,* **81,** 259–291.

Westoby, M., Falster, D.S., Moles, A.T., Vesk, P.A. & Wright, I.J. (2002) Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics,* **33,** 125-159.

Williamson, G.B. & Wiemann, M.C. (2010) Measuring wood specific gravity... Correctly. *American Journal of Botany,* **97,** 519–524.

Woodruff, D.R. & Meinzer, F.C. (2011) Water stress, shoot growth and storage of non-structural carbohydrates along a tree height gradient in a tall conifer. *Plant, Cell and Environment,* **34,** 1920–1930.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C., Falster, D.S., Garnier, E., Hikosaka, K., Lamont, B.B., Lee, W., Oleksyn, J., Osada, N., Poorter, H., Villar, R., Warton, D.I. & Westoby, M. (2005a) Assessing the generality of global leaf trait relationships. *New Phytologist,* **166,** 485–496.

Wright, I.J., Reich, P.B., Cornelissen, J.H.C., Falster, D.S., Groom, P.K., Hikosaka, K., Lee, W., Lusk, C.H., Niinemets, U., Oleksyn, J., Osada, N., Poorter, H., Warton, D.I. & Westoby, M. (2005b) Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography,* **14,** 411-421.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W., Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, U.l., Oleksyn, J., Osada, N., Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker, M.G., Veneklaas, E.J. & Villar, R. (2004) The world-wide leaf economics spectrum. *Nature,* **428,** 821–827.

Wright, I.J., Westoby, M. & Reich, P.B. (2002) Convergence towards higher leaf mass per area in dry and nutrient-poor habitats has different consequences for leaf lifespan. *Journal of Ecology,* **90,** 534–543.

Wright, S.J., Kitajima, K., Kraft, N.J.B., Reich, P.B., Wright, I.J., Bunker, D.E., Condit, R., Dalling, J.W., Davies, S.J., DÍAz, S., Engelbrecht, B.M.J., Harms, K.E., Hubbell, S.P., Marks, C.O., Ruiz-Jaen, M.C., Salvador, C.M. & Zanne, A.E. (2010) Functional traits and the growth–mortality trade-off in tropical trees. *Ecology,* **91,** 3664-3674.

Würth, M.K.R., Pelaez-Riedl, S., Wright, S.J. & Körner, C. (2005) Non-structural carbohydrate pools in a tropical forest. *Oecologia,* **143,** 11–24.

Xiang, S., Reich, P.B., Sun, S. & Atkin, O.K. (2013) Contrasting leaf trait scaling relationships in tropical and temperate wet forest species. *Functional Ecology,* **27,** 522–534.

Ziemińska, K., Westoby, M. & Wright, I.J. (2015) Broad Anatomical Variation within a Narrow Wood Density Range—A Study of Twig Wood across 69 Australian Angiosperms. *PLoS ONE,* **10,** e0124892.

3 CHAPTER III

MAINTENANCE PRUNING OF URBAN TREES DOES NOT INDUCE CARBOHYDRATE DEPLETION IN NORWAY MAPLE OR SILVER MAPLE

Jorge A. Ramirez[1], Tanya Handa[1] , Juan M. Posada[2], Sylvain Delagrange [3] and Christian Messier[1,3]

[1]Center for Forest Research, Université du Québec à Montréal, P.O. Box 8888, Succursale Centre-ville, Montréal, Québec, H3C 3P8, Canada

[2]Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá, Colombia

[3]Institute of Temperate Forest Sciences (ISFORT), University of Quebec in Outaouais (UQO), 58 Rue Principale, Ripon, QC, J0V 1V0, Canada

In preparation

Key words: Non-structural carbohydrates, compensatory mechanisms, urban stresses, shade intolerance/tolerance, *Acer platanoides*, *Acer saccharinum*.

3.1 ABSTRACT

Carbon allocation to reserves is an important trait that contributes to a plant's ability to tolerate stress. We studied two common urban tree species in northeastern North America, *Acer saccharinum* (Silver maple, native) and *Acer platanoides* (Norway maple, exotic) to assess the dynamics of non-structural carbohydrate (NSC) concentrations immediately following a maintenance pruning of 20 to 30% removal of the tree crown. NSC concentrations were measured by high-performance liquid chromatography in branch, main stem and root tissues for both pruned and un-pruned trees at three intervals during the growing season. NSC concentrations in tree organs of *A. platanoides* were 75% more than in *A. saccharinum*. Maintenance pruning did not have any significant depletion effect on the carbohydrate concentrations of either species and un-pruned branches close to pruned branches did not suffer any carbohydrate depletion. Yet, there was a significant temporal response of branches to pruning that differed between species. NSC concentrations in unpruned branches of pruned trees of A. platanoides increased at the end of the growing season, while no effect was measured in *A. saccharinum*. Higher levels of carbohydrates after pruning suggest that *A. platanoides* has compensatory mechanisms that allow this species to respond better to urban maintenance pruning stress than *Acer saccharinum*.

3.2 INTRODUCTION

Trees coordinate the allocation of carbon between support tissues, defense, and storage (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013). The storage pool is comprised mainly of non-structural carbohydrates (NSC) that can be mobilized to maintain plant metabolism during periods of unfavorable conditions for plant growth (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013). In trees, the most important NSC are low molecular weight sugars (glucose, fructose, sucrose) and starch (Chapin, Schulze & Mooney 1990; Hoch, Richter & Körner 2003), although some oligosaccharides and sugar alcohols also may be important storage compounds in certain species (Hoch, Richter & Körner 2003). In general, low weight sugars and sugar alcohols are used for short-term metabolism, while starch is stored in a more recalcitrant form for long-term use (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013).

The maintenance of carbohydrate reserves in potential storage pools (e.g. woody tissues) of trees is necessary to support metabolic requirements and compensatory growth after periods of heavy demand for carbohydrates (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013). Overall, plants with functional strategies adapted to cope with low-light environments (shade tolerant species) have a conservative carbon strategy that allocates proportionally more resources to defense and storage at the expense of reduced growth rates (Kobe 1997; Walters & Reich 1999; Myers & Kitajima 2007). In contrast, shade intolerant species have a higher relative carbon investment in growth and lower in storage because competition is one of the main selective filters in high–light environments (Kitajima 1994). Thus, there is a link between the carbohydrate storage and the light requirements of the species that may determine the growth and survival of trees (Myers & Kitajima 2007; Poorter & Kitajima 2007).

NSC reserves are located mainly in branches, stems, and coarse roots, because these woody perennial organs constitute the largest proportion of tree biomass. The concentrations of NSC in these tissues fluctuate due to mobilization and subsequent replenishment during the year, which depends in turn on the seasonal dynamics of NSC concentrations and the carbon source-sink balance between organs (Kozlowski 1992). Generally, minimum NSC concentrations occur in spring when storage pools mobilize NSC to sinks to support tissue growth and respiration, and maximum NSC concentrations are attained in autumn after the growing season when storage pools are replenished (Barbaroroux & Bréda 2002; Hoch, Richter & Körner 2003; Palacio, Maestro & Montserrat-Martí 2007).

The removal of plant tissues (as in the case of defoliation and pruning) can modify the source-sink balance between organs. This causes changes in NSC concentrations depending on the functional role of the damaged organs (sources or potential sinks) and the time at which removal occurs. On one hand, the removal of photosynthesizing biomass causes a reduction in carbohydrate synthesis, such as in the case of defoliation. On the other hand, the removal of vegetative sinks leads to a reduction in carbohydrate concentrations due to the mobilization of NSC to support metabolic demand and compensatory growth (Li, Hoch & Körner 2002; Handa, Körner & Hättenschwiler 2005; Palacio *et al.* 2008; Mei *et al.* 2015). Also, the time of the year in which the removal occurs determines the level of reserves available for tree recovery, according to the seasonal dynamics of NSC concentrations (Johnson 2007).

Overall, after tissue removal, carbohydrates are supplied from the closest sources at the expense of the more distant ones (Münch mass-flow theory of assimilate

transport, Wardlaw 1990; Le Roux *et al.* 2001). For example, a steep reduction of carbohydrate reserves in branches after bud break indicates a strong dependence on the closest carbon sinks (Hoch, Richter & Körner 2003; Landhäusser & Lieffers 2012). Branches from some deciduous trees may be carbon autonomous and do not drain stored carbohydrates from other parts of the tree, even when they are subjected to heavy stress, as in the case of photosynthetic tissue removal (Sprugel, Hinckley & Schaap 1991; Sprugel 2002; Hoch 2005).

Trees growing in urban areas are confronted with multiple anthropogenic stresses that may cause a loss of vitality by increasing their susceptibility to pathogenic organisms or reduce their rate of growth and longevity (Nilsson, Randrup & Wandall 2000; Mittler 2006). One of the most common urban stressors is branch pruning to control plant size and improve tree appearance (Clark & Matheny 2010). Higher carbohydrate reserves and rapid replenishment of carbohydrates should be important in allowing trees to respond appropriately to urban stressors, such as pruning. Nevertheless, NSC concentration has not been reported as a factor contributing to the success of urban species, and relatively little is known about the effect that urban tree pruning has on the dynamics of carbohydrate reserves.

We measured the seasonal dynamics of NSC in branches, stems, and roots for both pruned and un-pruned trees of *Acer saccharinum* (shade-intolerant) and *Acer platanoides* (shade tolerant), two common species of urban forests of eastern North America, to evaluate the dynamics of NSC concentrations after pruning during a single growing season. We hypothesized that (*i*) shade-tolerant *A. platanoides* maintains a higher carbohydrate concentration in reserves than the shade-intolerant *A. saccharinum*; (*ii*) maintenance pruning causes a depletion in carbohydrate reserves for both species following treatment; and (*iii*) the carbohydrate depletion is greater for un-pruned branches close to pruned branches compared to other tissues situated

further away from where pruning occurred such as stem and roots.

3.3 METHOD

3.3.1 Study site, selected species and pruning treatment

The study was conducted in a residential neighborhood in the city of Montreal (Quebec, Canada). We studied *A. saccharinum* L. (Silver maple) and *A. platanoides* L. (Norway maple). *A. saccharinum* is a native, intermediate to shade-intolerant species (Burns & Honkala 1990), whereas *A. platanoides* is a more shade-tolerant species that was brought from Europe to America as an ornamental tree (Nowak & Rowntree 1990). *A. platanoides* has been reported as being widely adapted to conditions in eastern North America; it has the capacity to tolerate higher stress levels than native flora (including its native congeners *Acer saccharinum and Acer saccharum*) due to certain ecophysiological advantages, such as its long seasonal growth and phenotypic plasticity (Martin & Marks 2006; Lapointe & Brisson 2012; Paquette *et al.* 2012).

The trunks of selected trees were located about 2 - 3 m from pavement, between street and sidewalk, or in front yards immediately adjacent to sidewalks. We sampled trees with similar heights (13.9 m on average) and diameters (63.2 cm average DBH) that appeared healthy, with no signs of physical damage or presence of pathogens. Both species are pruned periodically to control plant size and reduce the risk of short circuits caused by branches touching electrical lines. Pruning of trees was done by the local energy distribution company (Hydro-Quebec) in November 2010. Pruning consisted of removing the branches at the center of the tree, directly below the power lines in a "V shape" (Figure 3.1). The biomass removed by pruning was quantified by LiDAR scans and represented 20-30% of total branch biomass (Lecigne 2013) .

3.3.2 Tree sampling

For both species, five trees with pruning and five without the pruning treatment were sampled (20 trees in total) in April 2011 before bud break, in late June 2011 at the peak of shoot growth and in October 2011 at the end of the growing season. At the time of the first sampling, buds of both species were still dormant. Leaves started to expand in the middle of May and by the end of June they were fully expanded and hardened. By the end of October, leaves started to fall; however, leaves of *A. platanoides* stayed on the tree for a few days longer than leaves of *A. saccharinum.*

Samples of roots, stems, and branches were collected from all trees using a 2 mm increment puncher, which minimized injuries (Figure 3.1). Root samples were taken on surface roots ca. 50 cm away from the main stem. Stem samples were taken at 1.3 m height and at the first branch fork (first fork samples). In addition, two branch samples were taken from pruned trees, one sample from an un-pruned branch and a second sample from the pruned branch close to the wound. In unpruned trees, one sample was taken per tree from healthy branches at a height similar to the location sampled in pruned trees. Samples were placed in paper bags in the field, microwaved in the lab, and oven-dried at 65 °C.

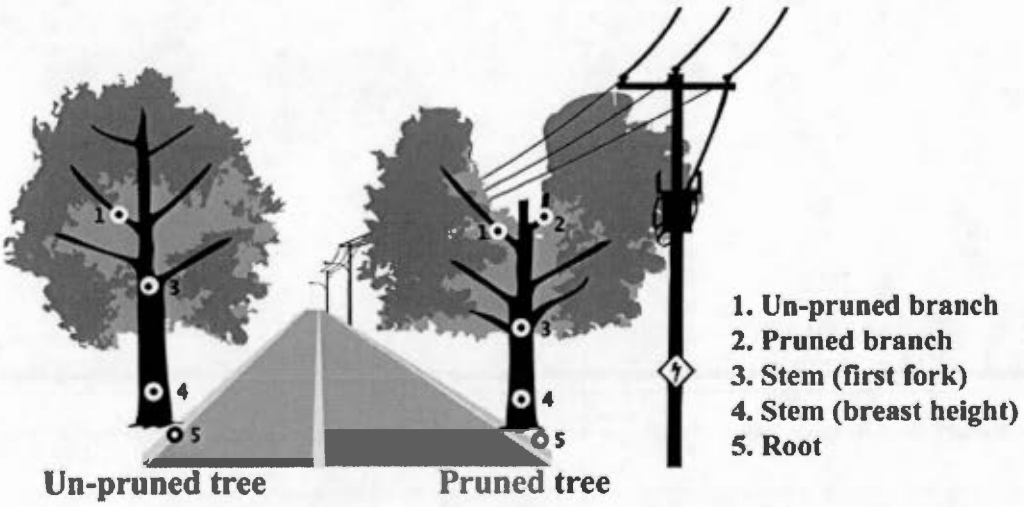

Figure 3.1 Schematic representation of the tree sampling

Left: Un-pruned tree. Right: Pruned tree. Points indicate the location of the samples collected from each tree during the 2011 growing season.

3.3.3 Components of non-structural carbohydrates and starch analysis

Samples were analyzed for low molecular weight sugars (fructose, glucose, sucrose), other sugars (oligosaccharides (raffinose), sugar alcohols (myo-inositol and sorbitol), and starch. The sum of low molecular weight sugars, other sugars, and starch is referred to here as non-structural carbohydrates (NSC). For sugars, 20 mg of tissue were treated with a methanol:chloroform mixture (12:5) in a 60 °C water bath for 30 minutes. An aliquot of the aqueous phase was vacuum-dried, stabilized with BSTFA (N, O-bis (trimethylsilyl) trifluoroacetamide)) and TMCS (trimethylchlorosilane), and analyzed on a gas chromatograph/mass spectrometer Varian 3800/Saturn 2000™ using MS WS software (Walnut Creek, CA, USA). Phenyl-glucopyranoside was used as the internal standard. For analysis of starch, we treated 50 mg of tissue with 80% ethanol at 95 °C. The tissue remaining after the ethanol extraction was digested with α-amylase and amyloglucosidase and then measured colorimetrically at an absorbance of 525 nm (Chow & Landhäusser 2004).

3.3.4 Data analysis and statistical analysis

We fitted linear mixed models to test the effect of pruning on carbohydrate concentrations over the study period. Models considered sugars (low weight sugars and other sugars), starch, and NSC as response variables. Tissue, species, time, and treatment (control and pruning) were considered as fixed factors, and individual trees were treated as random factors. Best models were selected according to the Akaike Information Criteria (AIC, lower AIC indicates a better model), which considers the fit and complexity of the model. Likelihood ratio tests (L) were also used to evaluate the effect of fixed factors, because the ANOVA method is more sensitive to the order of the terms and unbalanced data (Zuur *et al.* 2009). Effects were considered significant at $P < 0.05$. Finally, differences in mean concentration responses were

assessed using multiple comparisons of means (Tukey's tests). Analyses were performed using the ''lme'' function from the package ''nlme'' in the program R 3.1.1 (R_Core_Team 2013; Pinheiro *et al.* 2015).

3.4 RESULTS

3.4.1 Species differences in carbohydrate concentrations

Overall, throughout the growing season, carbohydrate concentrations in the tree organs of *A. platanoides* were 75% higher than in *A. saccharinum* (Figure 3.2, Table 3.1). The contribution of the different carbohydrates to total NSC was relatively similar in both species (Figure 3.2, Annex 1). Low weight sugars were the most common component of NSC, and accounted for 60-89% during the three sampling periods, followed by starch (5-36%) and other sugars (1-15%) (Figure 2, Annex 1). Also, NSC and low weight sugars followed the same seasonal pattern in both species, except for other sugars where a species-specific response was observed (Table 3.1). NSC and low weight sugars decreased from a high concentration in April to a lower concentration (in some sugars such as myo-inositol close to zero) in June, and then increased to the highest concentration in October. In contrast, starch increased continuously during the growing season (Figure 3.2).

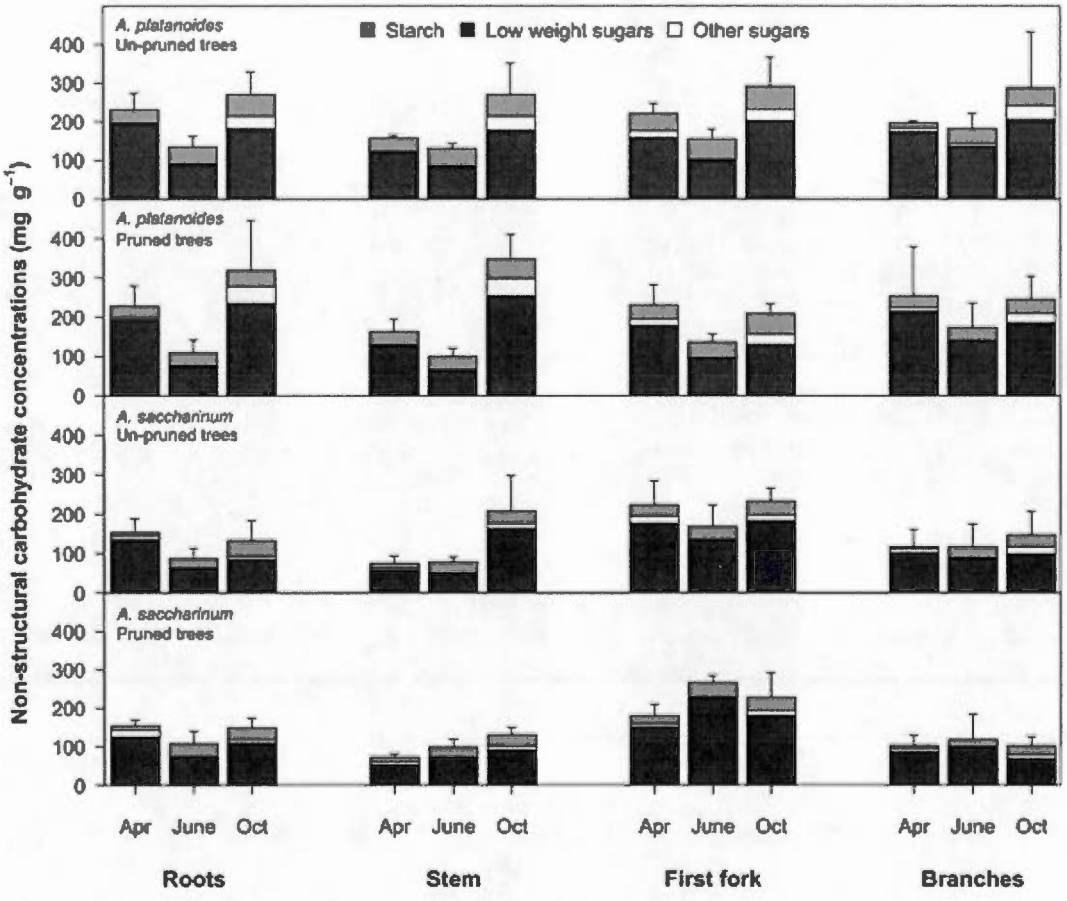

Figure 3.2 Seasonal dynamics of carbohydrate concentrations in un-pruned and pruned (excluding un-pruned branches of pruned trees) trees of *Acer saccharinum* and *Acer platanoides.*

Mean NSC concentrations (with standard errors) shown are the sum of starch, low weight sugars, and other sugars.

Table 3.1 Parameters of selected mixed effects models for time, tissue, species, and pruning treatment and their interactions on low weight sugars, other sugars, starch and non-structual carbohydrate concentrations in urban trees of *Acer saccharinum* and *Acer platanoides*. The analysis excludes unpruned branches of pruned trees in both speccies.

Parameter	LWS		OS		Starch		NSC	
	t-value	*P*-value	*t*-value	*P*-value	*t*-value	*P*-value	*t*-value	*P*-value
Time	1.73	0.09	**9.31**	**<0.001**	**9.64**	**<0.001**	**3.84**	**<0.001**
Tissue	-1.47	0.14			1.77	0.08		
Species	**-4.78**	**<0.001**	**4.73**	**<0.001**	**-4.15**	**<0.001**	**-6.61**	**<0.001**
Species*Time			**-7.18**	**<0.001**				

P values < 0.05 are in bold. NSC is the sum of starch, low weight sugars, and other sugars. See Annex 2 for all models considered.

3.4.2 Effect of pruning on carbohydrate concentrations and differences by tissue

We found no effect of pruning or of tissue type on carbohydrate concentrations when we compared carbohydrate concentrations of pruned (excluding un-pruned branches) and unpruned trees of both species (Table 3.1, Annex 2). However, when we included un-pruned branches from pruned trees (Figure 3.1) and analyzed branches separately, we found a significant effect of species, treatment, and time on the concentrations of low weight sugars and NSC (Table 3.2, Annex 3). Also, the interactions between species, treatment, and time were significant, indicating that the response to treatment was species-specific and time-specific in branches. Overall, in unpruned branches of pruned trees of *A. platanoides,* the concentrations of low weight sugars and NSC increased significantly at the end of the growing season compared to pruned branches and unpruned branches from unpruned trees. Unlike *A. platanoides*, carbohydrate concentrations in branches of *A. saccharinum* did not show any significant difference in the concentrations among branches (multiple comparisons of means, $P < 0.05$, Figure 3.3).

Table 3.2 Parameters of selected mixed effects models for time, species, pruning treatment and their interactions on low weight sugars, other sugars, starch and non-structual carbohydrate concentrations in urban trees of *Acer saccharinum* and *Acer platanoides*. The analysis includes both pruned and unpruned branches of un-pruned and pruned trees in both species.

Parameter	LWS		OS		Starch		NSC	
	t-value	*P*-value	*t*-value	*P*-value	*t*-value	*P*-value	*t*-value	*P*-value
Time	**-2.11**	**<0.05**	**5.10**	**<0.001**	**8.31**	**<0.001**		
Species	**-2.19**	**<0.05**	**2.48**	**<0.05**	**-4.67**	**<0.001**	**-2.09**	**<0.05**
Pruning	**-2.97**	**<0.001**					**-2.60**	**<0.01**
Species*Time			**-3.69**	**<0.001**				
Species*Pruning	**2.61**	**<0.01**					**2.34**	**<0.05**
Pruning*Time	**3.72**	**<0.001**					**3.28**	**<0.001**
Species*Pruning*Time	**-3.23**	**<0.01**					**-2.89**	**<0.01**

NSC is the sum of starch, low weight sugars, and other sugars. See Annex 3 for all models considered.

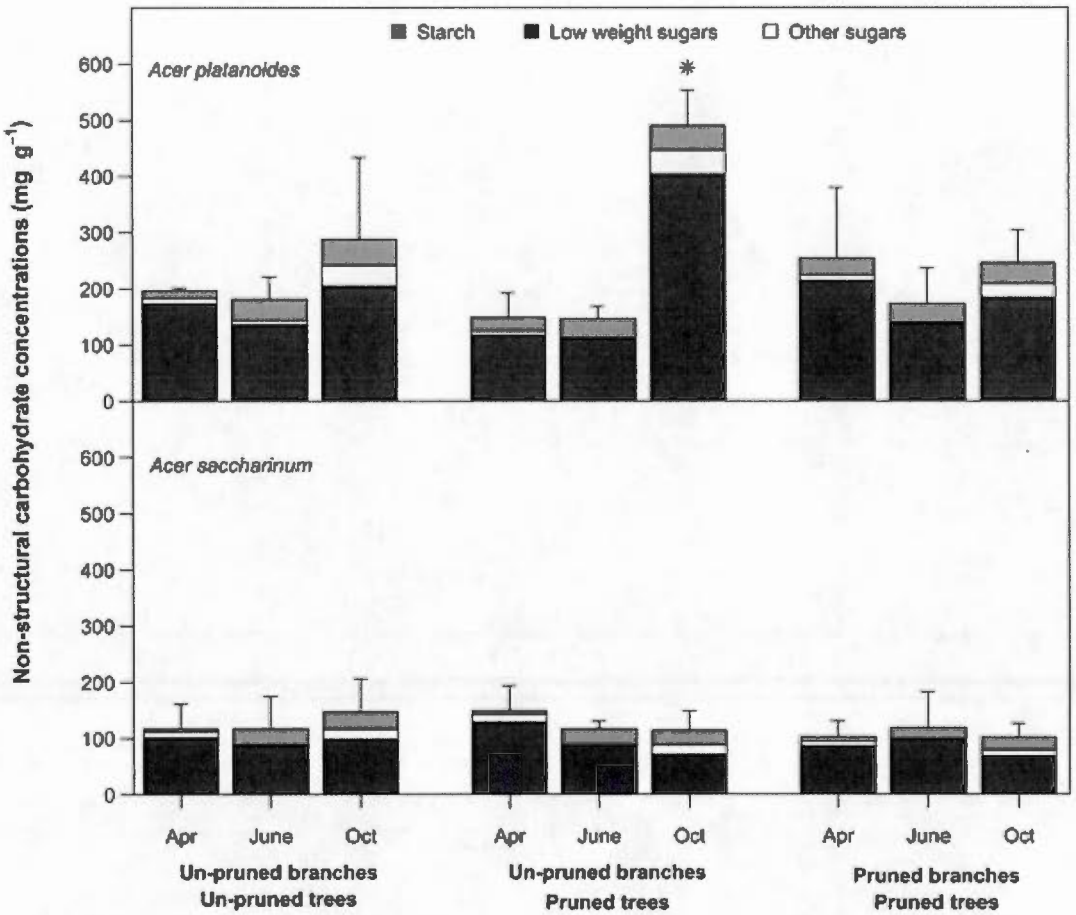

Figure 3.3 Mean concentrations of carbohydrates (with standard errors) are shown in branches from un-pruned and pruned trees (un-pruned and pruned) of Acer saccharinum and Acer platanoides during the growing season. NSC concentration is the sum of starch, , low weight sugars, and other sugars. * P < 0.05

3.5 DISCUSSION

3.5.1 Species differences in carbohydrate concentrations

As hypothesized in this study, and shown in other studies (Canham *et al.* 1999; Gaucher *et al.* 2005), the more shade-tolerant *A. platanoides* displayed higher carbohydrate concentrations than the shade-intolerant *A. saccharinum* throughout the growing season (Figure 3.2). It has been shown that shade-tolerant species allocate higher quantities of carbon to reserves and defense at the expense of growth than do light-demanding species (Kobe 1997; Myers & Kitajima 2007; Piper 2015). This higher allocation to reserves plays an important role in the growth and survival of trees, because it increases resilience and reduces the risk of mortality during periods of negative carbon balance. For example, high NSC concentrations have been shown to play a role in the recovery of plants after drought (McDowell *et al.* 2008; Mitchell *et al.* 2013; O'Brien *et al.* 2014), cold tolerance (Wong, Baggett & Rye 2003), as well as for protection from pathogens and insects (Kobe 1997; Canham *et al.* 1999; Myers & Kitajima 2007; Atkinson *et al.* 2014). Clearly, such high levels of carbohydrate reserves, combined with a high overall light interception capacity and other growth differences (Martin & Marks 2006; Lapointe & Brisson 2012; Paquette *et al.* 2012) provide *A. platanoides* with a net advantage over *A. saccharinum* in coping with urban pruning maintenance stress.

Seasonal patterns of concentrations of soluble sugars were different than those for starch concentrations in all plant tissues in both species. In general, low weight sugars decreased concentrations in the middle of the growing season, and then reached high levels by the end of the growing season where they remained until the end of dormancy (Wong, Baggett & Rye 2003; Gaucher *et al.* 2005; Carbone *et al.* 2013). An initial decrease in sugar concentrations may be due to the fact that photosynthates

produced at the beginning of the growing season are not stored, but are mobilized to satisfy growth and other metabolic needs. Then, in the middle of the summer and the months that follow, the products of photosynthesis are allocated to reserves and carbohydrate concentrations increase due to the reduction in sink strength (Wong, Baggett & Rye 2003; Gaucher *et al.* 2005). In contrast, starch increased continuously during the growing season and achieved a maximum in fall before the beginning of dormancy. Although we did not measure winter dynamics, NSC concentrations typically decrease in winter when starch is hydrolyzed and converted to free sugars for cold tolerance (Wong, Baggett & Rye 2003).

3.5.2 Effect of pruning on carbohydrate concentrations

The source-sink imbalance generated by pruning did not deplete the carbohydrate reserves when we compared tissues from un-pruned and pruned trees in both species (treatment effect was not significant, Table 3.1). This fact may be explained by the intensity of the perturbation, which modulated the response of carbohydrate concentrations to lost tissue (Fang *et al.* 2006; Eyles, Pinkard & Mohammed 2009; Quentin *et al.* 2011; Atkinson *et al.* 2014). In this case, the percentage of biomass removed by pruning was not enough to cause any depletion of carbohydrate concentrations due to metabolic demand and compensatory growth. In fact, one year following pruning, trees had recovered their crown totally without any sign of a reduction in growth (Lecigne 2013). These results were surprising and contrary to expectations, because our pruning treatment was close to the pruning limits proposed by the American National Standards Institute to maintain the health of urban trees (ANSI 2001; Gilman 2002; Johnson 2007). Current guidelines suggest that urban trees, unlike their counterparts in the forest that may tolerate higher pruning intensities (Wadsworth 1997; Pinkard & Beadle 2000; James 2004), should not be

pruned more than 25% to prevent insect attacks, disease, or even death under unfavorable conditions for growth in urban areas (ANSI 2001).

Several studies have investigated the effect of disturbance intensity/severity on the concentrations of carbohydrates. Overall, when the intensity of the perturbation of defoliation due to pruning is intermediate as in this study, carbohydrate concentrations are not altered (Chesney & Vasquez 2007; Palacio *et al.* 2008; Barry *et al.* 2012), especially in slow-medium growing species (Canham *et al.* 1999; Atkinson *et al.* 2014). This lack of a pruning effect on carbohydrate concentrations may indicate a high carbon loading of trees, and supports the idea that they tend not to be limited by carbon supply (Hoch, Richter & Körner 2003; Körner 2003; Würth *et al.* 2005). This result may also suggest that new foliar resources from photosynthesis meet the demand for tree recovery and may be more important than carbon reserves that are stored in farther plant tissues (Körner 2003; Barry *et al.* 2012).

3.5.3 Effect of pruning on plant tissues

Although we did not find any significant effect of pruning on the carbohydrate concentrations of tree tissues (Table 1), when we analyzed separately carbohydrate concentrations in branches from pruned trees (un-pruned and pruned branches) we found a significant effect of pruning (Table 3.2). Initially we expected a depletion in carbohydrate concentrations in un-pruned branches on pruned trees due to the temporal demand for carbohydrates from branches to maintain metabolic activity and compensatory growth (carbon autonomy of branches, Landhäusser 2011; Landhäusser & Lieffers 2012). Contrary to what we expected, we found an increase of low weight sugars and NSC in un-pruned branches on pruned trees of *A. platanoides* at the end of the growing season (Table 3.2 and Figure 3.3). This increase in carbohydrates following pruning showed that urban trees of *A. platanoides*

maintained high quantities of reserves in above-ground biomass even after pruning, which is a recognized compensatory response related to tolerance to tissue loss (Strauss & Agrawal 1999; Eyles, Pinkard & Mohammed 2009). This strategy is typical of less shade-tolerant species (Handa, Körner & Hättenschwiler 2005; Atkinson *et al.* 2014) and shows that *A. platanoides* expresses strategies of both shade-tolerant and shade-intolerant species (Martin, Canham & Kobe 2010).

To conclude, although removal of more than 25% of live branches is not recommended in urban trees (ANSI 2001), we found that pruning levels of 20-30% did not have a significant effect on carbohydrate concentrations in either species. To improve planning and management operations on urban tree populations, it may be useful to determine which levels above these ranges induce a reduction in carbohydrate levels and a subsequent decrease in plant tolerance to environmental constraints, tree decline, and mortality (Palacio *et al.* 2008; Landhäusser & Lieffers 2012; Wiley *et al.* 2013; Saffell *et al.* 2014). Additionally, periodic assessment of the concentration of carbohydrate reserves in urban trees may suggest which species respond better to urban stressors and could provide data that may be used to enable source-sink models to better predict growth and survival after management treatments in urban trees.

3.6 ACKNOWLEDGEMENTS

This research was supported by the NSERC/Hydro-Quebec research chair on tree growth control. We thank Dylan Craven for critical feedback on the manuscript and Thomas Gavin for linguistic help. We are also grateful to Matt Follett for field assistance, and Marc Lucotte and Matthieu Moingt for their lab support .

3.7 SUPPORTING INFORMATION

3.7.1 Annex A

Table 3.3A Non-structural carbohydrates in urban trees of Acer platanoides and A. saccharinum (mg g-1 ± SE) at three times during the growing season 2011. Values are means and standard errors of five trees per species and treatment.

Species	Treatment	Tissue	LWS-t_1	LWS-t_2	LWS-t_3	OS-t_1	OS-t_2	OS-t_3	Starch-t_1	Starch-t_2	Starch-t_3	NSC-t_1	NSC-t_2	NSC-t_3
Norway Maple	Control	Roots	188.56 (20.48)	86.39 (18.84)	181.78 (28.39)	2.11 (0.42)	2.84 (0.99)	32.46 (8.42)	46.85 (13.52)	44.36 (11.22)	57.46 (5.25)	237.52 (28.56)	133.59 (15.41)	271.71 (33.67)
	Control	Stem	114.26 (7.11)	82.78 (9.97)	144.18 (49.98)	4.1 (3)	2.04 (0.61)	37.3 (6.25)	35.81 (4.07)	45.64 (5.98)	57.43 (3.98)	154.18 (3.96)	130.46 (7.17)	238.9 (49.65)
	Control	First Fork	158.54 (1.6)	98.02 (2.87)	202.79 (39.23)	16.72 (3.98)	3.51 (0.67)	25.33 (3.79)	46.42 (11.3)	54.09 (10.56)	61.35 (5.29)	221.68 (11.74)	155.61 (14.29)	289.47 (45.59)
	Control	Branches	175.04 (9.51)	134.44 (24.53)	204.55 (67.09)	7.32 (4.19)	6.87 (3.62)	32.41 (9.15)	14.11 (8.12)	38.9 (3.85)	47.23 (5.18)	196.47 (67.89)	180.22 (22.47)	284.19 (75.34)
	Pruning	Roots	187.7 (25.96)	72.72 (11.22)	235.89 (56.84)	5.63 (0.59)	2.74 (1.24)	39.24 (9.15)	29.39 (8.62)	33.65 (5.44)	42.31 (7.98)	222.72 (31.36)	109.12 (16.55)	317.45 (63.56)
	Pruning	Stem	125.6 (15.38)	62 (10.77)	244.34 (36.49)	2.56 (1.31)	1.83 (0.44)	38.89 (10.64)	35.75 (4.08)	35.58 (3.16)	51.55 (6.58)	163.91 (15.56)	99.41 (12.19)	334.78 (40.53)
	Pruning	First Fork	178.44 (26.05)	93.98 (9.77)	130.7 (14.43)	12.29 (6.92)	1.5 (0.53)	22.29 (1.29)	37.69 (9.13)	40.52 (1.56)	54.15 (4.03)	228.42 (29.56)	136 (10.56)	207.15 (32.43)
	Pruning	Branches	214.61 (76.64)	138.1 (30.53)	176.63 (23.87)	6.87 (2.33)	1.37 (0.22)	22.17 (5.98)	31.34 (7.69)	34.48 (4.28)	37.28 (6.98)	252.81 (73.61)	173.95 (31.78)	236.08 (36.2)
Silver Maple	Control	Roots	130.88 (16.43)	61.54 (15.29)	90.77 (20.34)	5.39 (2.75)	0.78 (0.28)	5.98 (0.89)	14.32 (6.95)	25.35 (8.8)	33.26 (10.5)	150.58 (21.56)	87.67 (15.48)	130.01 (30.29)
	Control	Stem	57.61 (8.24)	48.7 (4.4)	163.66 (49.59)	5.91 (3.27)	1.57 (0.9)	13.38 (4.39)	11.74 (0.92)	28.77 (5.76)	32.75 (4.57)	75.26 (10.93)	79.04 (4.31)	209.79 (53.56)
	Control	First Fork	176.62 (67.53)	132.73 (35.21)	182.3 (17.47)	17.93 (5.12)	1.67 (0.98)	11.68 (2.86)	29.3 (14.5)	34.74 (7.4)	37.48 (6.38)	223.85 (63.88)	169.14 (37.69)	231.47 (55.69)
	Control	Branches	98.72 (21.13)	85.43 (33.35)	95.26 (29.33)	7.87 (4.57)	2.64 (1.32)	15.59 (5.3)	6.17 (4.31)	29.01 (2.21)	31.53 (4.37)	112.75 (63.41)	117.07 (26.54)	142.38 (33.94)
	Pruning	Roots	126.09 (9.67)	72.11 (21.07)	108.88 (9.96)	15.32 (3.51)	1.53 (0.43)	9.32 (0.52)	13.16 (5.43)	34.98 (10.8)	33.24 (7.17)	154.56 (36.11)	108.61 (17.66)	151.44 (12.45)
	Pruning	Stem	53.27 (1.61)	71.33 (12.26)	91.47 (7.82)	7.54 (4.78)	0.98 (0.5)	11.2 (1.72)	12.71 (1.66)	26.91 (5.58)	30.14 (2.3)	73.52 (4.4)	99.23 (14.31)	132.81 (10.28)
	Pruning	First Fork	151.79 (27.39)	224.54 (6.91)	180.37 (35.76)	7.28 (0.76)	3.44 (1.9)	11.68 (2.97)	20.72 (1.89)	39.37 (5.77)	36.62 (3.94)	179.78 (30.42)	267.34 (3.98)	228.68 (50.98)
	Pruning	Branches	86.48 (12.34)	97.19 (31.68)	68.6 (12.48)	7.71 (1.88)	1.95 (0.42)	8.6 (1.95)	8.8 (3.96)	19.98 (3.69)	24.17 (2.63)	102.99 (14.51)	119.12 (32.72)	101.37 (13.59)

LWS: Low weight sugars. OS: Other sugars. NSC is the sum of starch, low weight sugars and other sugars.

3.7.2 Annex B

Table 3.4B Full set of linear mixed models considered with the Akaike information criterion (AIC), likelihood ratio test estimates (L), and their respective inference (P value).

Analysis for tissue, time, species, and pruning treatment and their interactions on non-structual carbohydrate (NSC) concentrations in urban trees of *Acer saccharinum* and *Acer platanoides*. Analyses exclude un-pruned branches of pruned trees in both species

Model	df	LWS			OS			Starch			NSC		
		AIC	*L*	*P*-value	AIC	*L*	*P*-value	AIC	*L*	*P*-value	AIC	*L*	*P*-value
~ 1	3	2748.45			1921.50			2010.56			2825.15		
Tissue	4	2748.35	2.10	0.15	1923.40	0.10	0.75	2010.35	2.21	0.14	2825.57	1.58	0.21
Species	4	2732.29	18.16	<.001	1907.08	16.42	<.001	1999.97	12.59	<.001	2801.30	25.85	<.001
Time	4	2747.53	2.92	0.09	1878.56	44.94	<.001	1934.93	77.62	<.001	2812.96	14.19	<.001
Pruning	4	2750.39	0.06	0.81	1923.34	0.17	0.68	2012.00	0.56	0.45	2827.15	0.00	1.00
Tissue + Species	5	2732.13			1908.97			1999.76			2801.68		
Tissue * Species	6	2734.08	0.05	0.82	1910.75	0.22	0.64	2001.70	0.06	0.80	2803.67	0.01	0.92
Tissue + Time	5	2747.41			1880.43			1933.78			2813.27		
Tissue * Time	6	2749.03	0.37	0.54	1881.02	1.41	0.23	1935.51	0.27	0.60	2814.14	1.13	0.29
Tissue + Pruning	5	2750.29			1925.23			2011.79			2827.57		
Tissue * Pruning	6	2752.08	0.21	0.64	1923.51	3.73	0.05	2013.65	0.14	0.71	2829.39	0.18	0.67
Species + Time	5	2731.29			1862.16			1924.35			**2788.81**		
Species * Time	6	2732.79	0.50	0.48	**1816.69**	47.47	<.001	1926.22	0.13	0.72	2789.05	1.76	0.18

Model	df	LWS			OS			Starch			NSC		
		AIC	*L*	*P*-value	AIC	*L*	*P*-value	AIC	*L*	*P*-value	AIC	*L*	*P*-value
Species + Pruning	5	2734.18			1908.86			2000.91			2803.30		
Species * Pruning	6	2736.10	0.09	0.77	1910.83	0.03	0.86	2002.50	0.41	0.52	2805.25	0.05	0.83
Time + Pruning	5	2749.47			1880.39			1936.38			2814.96		
Time * Pruning	6	2751.37	0.10	0.75	1882.33	0.06	0.81	1935.00	3.37	0.07	2816.33	0.63	0.43
Tissue + Species + Time	6	**2731.11**			1864.02			1923.20			**2789.09**		
Tissue * Species * Time	10	2737.54	1.57	0.81	1823.78	58.24	<.001	1930.16	1.04	0.90	2792.59	4.50	0.34
Tissue + Species + Pruning	6	2734.02			1910.75			2000.70			2803.68		
Tissue * Species * Pruning	10	2741.61	0.41	0.98	1914.42	4.32	0.36	2005.90	2.80	0.59	2811.39	0.29	0.99
Tissue + Time + Pruning	6	2749.35			1882.27			1935.22			2815.27		
Tissue * Time * Pruning	10	2754.68	2.67	0.61	1883.65	6.61	0.16	1939.03	4.19	0.38	2818.37	4.91	0.30
Species * Time * Pruning	6	2733.18			1863.88			1925.29			2790.81		
Species + Time + Pruning	10	2740.49	0.70	0.95	1824.08	47.80	<.001	1928.73	4.55	0.34	2796.27	2.54	0.64
Tissue + Species + Time + Pruning	7	2733.00			1865.75			1924.14			2791.09		
Tissue * Species * Time * Pruning	18	2749.56	5.44	0.91	1820.07	67.68	<.001	1936.10	10.04	0.53	2801.83	11.27	0.42

Selected models are in bold (see Table 1 for parameter statistics of best models selected).

3.7.3 Annex C

Table 3.5C Full set of linear mixed models considered with the Akaike information criterion (AIC), likelihood ratio test estimates (L), and their respective inference (P value).

Analysis for tissue, time, species, and pruning treatment and their interactions on non-structual carbohydrate (NSC) concentrations in urban trees of *Acer saccharinum* and *Acer platanoides*. Analysis includes branches from un-pruned and pruned trees (un-pruned and pruned) in both species.

Model	df	LWS			OS			Starch			NSC		
		AIC	*L*	*P*-value	AIC	*L*	*P*-value	AIC	*L*	*P*-value	AIC	*L*	*P*-value
~ 1	3	1082.33			735.01			738.05			1107.48		
Species	4	1061.96	22.38	<.001	732.64	4.37	<.05	725.60	14.45	<.001	1085.17	24.30	<.001
Time	4	1082.05	2.28	0.13	717.70	19.32	<.001	690.84	49.21	<.001	1103.47	6.01	<.05
Pruning	4	1083.67	0.66	0.42	736.99	0.02	0.88	740.02	0.03	0.86	1108.94	0.54	0.46
Species+Time	5	1061.34			714.25			**677.80**			1080.46		
Species*Time	6	1054.95	8.39	<.01	**703.00**	13.25	<.001	679.79	0.02	0.90	1074.79	7.68	<.01
Species+Pruning	5	1063.17			734.62			727.60			1086.54		
Species*Pruning	6	1064.37	0.80	0.37	736.58	0.03	0.85	729.46	0.14	0.71	1087.87	0.67	0.41
Time+Pruning	5	1083.37			719.67			692.67			1104.88		
Time*Pruning	6	1082.16	3.21	0.07	721.56	0.11	0.74	691.74	2.93	0.09	1104.59	2.29	0.13
Species+Time+Pruning	6	1062.53			716.22			679.75			1081.78		
Species*Time*Pruning	10	**1046.31**	24.22	<.001	709.75	14.47	<.01	684.25	3.50	0.48	**1069.71**	20.07	<.001

3.8 REFERENCES

ANSI (2001) *American National Standard for tree care operations—Tree, Shrub, and Other Woody Plant Maintenance—Standards practices (Pruning). ANSI A300 (part 1)*. American National Standards Institute, New York.

Atkinson, R.R.L., Burrell, M.M., Rose, K.E., Osborne, C.P. & Rees, M. (2014) The dynamics of recovery and growth: how defoliation affects stored resources. *Proceedings of the Royal Society B,* **281,** 20133355.

Barbaroroux, C. & Bréda, N. (2002) Contrasting distribution and seasonal dynamics of carbohydrate reserves in stem wood of adult ring-porous sessile oak and diffuse-porous beech trees. *Tree Physiology,* **22,** 1201–1210.

Barry, K.M., Quentin, A., Eyles, A. & Pinkard, E.A. (2012) Consequences of resource limitation for recovery from repeated defoliation in Eucalyptus globulus Labilladière. *Tree Physiology,* **32,** 24-35.

Burns, R.M. & Honkala, B.H. (1990) *Silvics of North America Volume 2, Hardwoods. Agriculture Handbook 654*. USDA Forest Service, Washington.

Canham, C.D., Kobe, R.K., Latty, E.F. & Chazdon, R.L. (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. *Oecologia,* **121,** 1-11.

Carbone, M.S., Czimczik, C.I., Keenan, T.F., Murakami, P.F., Pederson, N., Schaberg, P.G., Xu, X. & Richardson, A.D. (2013) Age, allocation and availability of nonstructural carbon in mature red maple trees. *New Phytologist,* **200,** 1145–1155.

Clark, J.R. & Matheny, N. (2010) The Research Foundation to Tree Pruning: A Review of the Literature. *Arboriculture & Urban Forestry,* **36,** 110–120.

Chapin, F.S., Schulze, E.D. & Mooney, H.A. (1990) The ecology and economics of storage in plants. *Annual Review of Ecology and Systematics,* **21,** 423-447.

Chesney, P. & Vasquez, N. (2007) Dynamics of non-structural carbohydrate reserves in pruned Erythrina poeppigiana and Gliricidia sepium trees. *Agroforestry Systems,* **69,** 89–105.

Chow, P.S. & Landhäusser, S.M. (2004) A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology,* **24,** 1129–1136.

Dietze, M.C., Sala, A., Carbone, M.S., Czimczik, C.I., Mantooth, J.A., Richardson, A.D. & Vargas, R. (2013) Nonstructural Carbon in Woody Plants. *Annual Review of Plant Biology,* **65,** 2.1–2.21.

Eyles, A., Pinkard, E.A. & Mohammed, C. (2009) Shifts in biomass and resource allocation patterns following defoliation in Eucalyptus globulus growing with varying water and nutrient supplies. *Tree Physiology,* **29,** 753–764.

Fang, X.W., Yuan, J.L., Wang, G. & Zhao, Z.G. (2006) Fruit production of shrub, Caragana korshinskii, following aboveground partial shoot removal: mechanisms underlying compensation. *Plant Ecology,* **187,** 213–225.

Gaucher, C., Gougeon, S., Mauffette, Y. & Messier, C. (2005) Seasonal variation in biomass and carbohydrate partitioning of understory sugar maple (Acer saccharum) and yellow birch (Betula alleghaniensis) seedlings. *Tree Physiology,* **25,** 93–100.

Gilman, E.F. (2002) *An Illustrated Guide to Pruning*. Delmar Division, Thompson Learning, Albany, NY.

Handa, T., Körner, C. & Hättenschwiler, S. (2005) A test of the treeline carbon limitation hypothesis by in situ CO2 enrichment and defoliation. *Ecology,* **86,** 1288-1300.

Hoch, G. (2005) Fruit-bearing branchlets are carbon autonomous in mature broad-leaved temperate forest trees. *Plant, Cell & Environment,* **28,** 651–659.

Hoch, G., Richter, A. & Körner, C. (2003) Non-structural carbon compounds in temperate forest trees. *Plant, Cell and Environment,* **26,** 1067-1081.

James, R. (2004) Plantation silviculture | High Pruning. *Encyclopedia of Forest Sciences* (ed. J. Burley). Elsevier, Oxford.

Johnson, D.L. (2007) Pruning. *Urban and Community Forestry in the Northeast* (ed. J.E. Kuser). Springer Netherlands, New York.

Kitajima, K. (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia,* **98,** 419-428.

Kobe, R.K. (1997) Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. *Oikos,* **80,** 226-233.

Körner, C. (2003) Carbon limitation in trees. *Journal of Ecology,* **91,** 4-17.

Kozlowski, T.T. (1992) Carbohydrate sources and sinks in woody plants. *Botanical Review,* **58,** 107-222.

Landhäusser, S.M. (2011) Aspen shoots are carbon autonomous during bud break. *Trees,* **25,** 531–536.

Landhäusser, S.M. & Lieffers, V.J. (2012) Defoliation increases risk of carbon starvation in root systems of mature aspen. *Trees,* **26,** 653–661.

Lapointe, M. & Brisson, J. (2012) A comparison of invasive Acer platanoides and native A. saccharum first-year seedlings: growth, Biomass distribution and the influence of ecological factors in a forest understory. *Forests,* **3,** 190-206.

Le Roux, X., Lacointe, A., Escobar-Gutiérrez, A. & Le Dizès, S. (2001) Carbon-based models of individual tree growth: a critical appraisal. *Annals of Forest Science,* **58,** 469–506.

Lecigne, B. (2013) Effets des tailles de dégagement des réseaux électriques sur la colonisation de l'espace par les arbres, développement et mise en application

d'une méthode d'analyse de données T-LiDAR. Master thesis. Master thesis, Université du Québec à Montréal (UQAM).

Li, M.H., Hoch, G. & Körner, C. (2002) Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees-Structure and Function,* **16,** 331-337.

Martin, P.H., Canham, C.D. & Kobe, R.K. (2010) Divergence from the growth–survival trade-off and extreme high growth rates drive patterns of exotic tree invasions in closed-canopy forests. *Journal of Ecology,* **98,** 778–789.

Martin, P.H. & Marks, P.L. (2006) Intact forests provide only weak resistance to a shade-tolerant invasive Norway maple (Acer platanoides L.). *Journal of Ecology,* **94,** 1070–1079.

McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G. & Yepez, E.A. (2008) Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytologist,* **178,** 719-739.

Mei, L., Xiong, Y., Gu, J., Wang, Z. & Guo, D. (2015) Whole-tree dynamics of non-structural carbohydrate and nitrogen pools across different seasons and in response to girdling in two temperate trees. *Oecologia,* **177,** 333–344.

Mitchell, P.J., O'Grady, A.P., Tissue, D.T., White, D.A., Ottenschlaeger, M.L. & Pinkard, E.A. (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytologist,* **197,** 862-872.

Mittler, R. (2006) Abiotic stress, the field environment and stress combination. *Trends in Plant Science,* **11,** 15-19.

Myers, J.A. & Kitajima, K. (2007) Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. *Journal of Ecology,* **95,** 383-395.

Nilsson, K., Randrup, T.B. & Wandall, B.M. (2000) Trees in the urban environment. *The Forest Handbook* (ed. J. Evans). Blackwell Science, Oxford.

Nowak, D.J. & Rowntree, R.A. (1990) History and range of Norway maple. *Journal of Arboriculture,* **16,** 291-296.

O'Brien, M.J., Leuzinger, S., Philipson, C.D., Tay, J. & Hector, A. (2014) Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate Change,* **4,** 710-714.

Palacio, S., Hester, A.J., Maestro, M. & Millard, P. (2008) Browsed *Betula pubescens* trees are not carbon-limited. *Functional Ecology,* **22,** 808-815.

Palacio, S., Maestro, M. & Montserrat-Martí, G. (2007) Seasonal dynamics of non-structural carbohydrates in two species of mediterranean sub-shrubs with different leaf phenology. *Environmental and Experimental Botany,* **59,** 34–42.

Paquette, A., Fontaine, B., Berninger, F., Dubois, K., Lechowicz, M.J., Messier, C., Posada, J.M., Valladares, F. & Brisson, J. (2012) Norway maple displays greater seasonal growth and phenotypic plasticity to light than native sugar maple. *Tree Physiology,* **32,** 1339-1347.

Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. (2015) nlme: Linear and Nonlinear Mixed Effects Models. *R package version 3.1-122.* R Core Team.

Pinkard, E.A. & Beadle, C.L. (2000) A physiological approach to pruning. *International Forestry Review,* **2,** 295-305.

Piper, F.I. (2015) Patterns of carbon storage in relation to shade tolerance in southern South American species. *American Journal of Botany,* **102,** 1442-1452.

Poorter, L. & Kitajima, K. (2007) Carbohydrate storage and light requirements of tropical moist and dry forest tree species. *Ecology,* **88,** 1000-1011.

Quentin, A.G., Beadlea, C.L., O'Gradya, A.P. & Pinkard, E.A. (2011) Effects of partial defoliation on closed canopy Eucalyptus globulus Labilladière: Growth, biomass allocation and carbohydrates. *Forest Ecology and Management,* **261,** 695-702.

R_Core_Team (2013) *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria.

Saffell, B.J., Meinzer, F.C., Woodruff, D.R., Shaw, D.C., Voelker, S.L., Lachenbruch, B. & Falk, K. (2014) Seasonal carbohydrate dynamics and growth in Douglas-fir trees experiencing chronic, fungal-mediated reduction in functional leaf area. *Tree Physiology.*

Sprugel, D.G. (2002) When branch autonomy fails: Milton's Law of resource availability and allocation. *Tree Physiology,* **22,** 1119-1124.

Sprugel, D.G., Hinckley, T.M. & Schaap, W. (1991) The theory and practice of branch autonomy. *Annual Review of Ecology, Evolution, and Systematics,* **22,** 309–334.

Strauss, S.Y. & Agrawal, A.A. (1999) The ecology and evolution of plant tolerance to herbivory. *Trends in ecology and evolution,* **14,** 179–185.

Wadsworth, F.H. (1997) *Forest production for tropical America.* United States Department of Agriculture, Washington, DC:.

Walters, M.B. & Reich, P.B. (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad leaved evergreen species differ? *New Phytologist,* **143,** 143–154.

Wardlaw, I.F. (1990) The control of carbon partitioning in plants. *New Phytologist,* **116,** 341–381.

Wiley, E., Huepenbecker, S., Casper, B.B. & Helliker, B.R. (2013) The effects of defoliation on carbon allocation: can carbon limitation reduce growth in favour of storage? *Tree Physiology,* **00,** 1-13.

Wong, B.L., Baggett, K.L. & Rye, A.H. (2003) Seasonal patterns of reserve and soluble carbohydrates in mature sugar maple (Acer saccharum). *Canadian Journal of Botany,* **81,** 780-788.

Würth, M.K.R., Pelaez-Riedl, S., Wright, S.J. & Körner, C. (2005) Non-structural carbohydrate pools in a tropical forest. *Oecologia,* **143,** 11–24.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) *Mixed Effects Models and Extensions in Ecology with R.* Springer, New York.

4 CHAPTER IV

SINGLE AND COMBINED EFFECTS OF EXPERIMENTAL DEFOLIATION, ROOT PRUNING, AND STEM GIRDLING ON CARBOHYDRATE RESERVES AND GROWTH IN THREE COMMON NORTH AMERICAN URBAN TREE SPECIES

Jorge A. Ramirez[1], Tanya Handa[1], Juan M. Posada[2] and Christian Messier[1,3]

[1]Center for Forest Research, Université du Québec à Montréal, P.O. Box 8888, Succursale Centre-ville, Montréal, Québec, H3C 3P8, Canada

[2]Facultad de Ciencias Naturales y Matemáticas, Universidad del Rosario, Bogotá, Colombia

[3]Institut des Sciences de la Forêt Tempérée (ISFORT), Université du Québec en Outaouais (UQO), Ripon, Quebec, Canada

In preparation

Keywords: non-structural-carbohydrates, storage, growth, stress, urban environment, Celtis occidentalis, Fraxinus pennsylvanica, Tilia cordata.

4.1 ABSTRACT

Trees that grow in urban areas are confronted with a wide variety of stresses that threaten their long-term survival. Some of these common stresses include crown damage, root reduction and stem injury. The single or combined effects of these stresses generate a complex array of growth and ecophysiological responses that are hard to predict. We hypothesized that (*i*) tree growth will be increasingly and negatively affected as stress levels increase; (*ii*) single and some combined stresses inflicted to the trees will negatively affect the levels of reserve concentrations found in tree tissues (roots, stems, branches and leaves) in order to maintain tree growth (*iii*) combined stresses will have a positive or negative impact on reserve concentrations depending on what eco-physiological mechanisms are being affected by the various stresses; and (*iv*) trees in stress treatments that result in higher carbohydrate concentrations would exhibit lower growth rates. To test these hypotheses, we set up a manipulative experiment using three common North American urban tree species (*Celtis occidentalis, Fraxinus pennsylvanica*, and *Tilia cordata*). These trees were submitted to an increasing level of single common stresses (three levels of defoliation and root pruning, and two levels of stem damage) and their combined effects under field conditions. As hypothesized, we found that tree growth declines in relation to the total amount of stress inflicted to the trees, i.e., when the combined highest level of stress was applied, but that contrary to our second hypothesis reserves were not affected or in some cases increased with increasing level of stress. We did not find a consistent response, contrary to our third hypothesis, in reserve concentrations in relation to the various combined stress treatments applied to the three tree species investigated. Finally, in agreement with our fourth hypothesis we found an inverse relationship between tree growth rate and reserve concentrations, suggesting that trees adjust their levels of carbohydrate, especially in stems and roots, to meet their

metabolic demand under stressful situations. Such acclimation appears to be an important mechanism allowing tree to increase their survivorship under different urban stress conditions.

4.2 INTRODUCTION

Trees are among the most valuable components of urban green areas due to their wide range of environmental, social, cultural, and economic benefits (Konijnendijk *et al.* 2005). Nevertheless, trees that grow in urban areas are confronted with a wide variety of biotic and abiotic stresses that can make their growing conditions harsher than that of trees that grow under natural conditions (Sieghardt *et al.* 2005). Such stresses include natural or introduced pathogens, insect defoliation, frost damage, and breakage by wind, which lead to defoliation and loss of woody tissues. Grey infrastructure often limits the space of trees to grow which combined with compact soils, and water and atmospheric pollution further exacerbate the problem (Konijnendijk & Randrup 2004; Tubby & Webber 2010). Additionally, other stress such as girdling and ring-barking of trees often occur from vehicle impact, lawn mowers, weed trimmers and human vandalism (Moore 2013; Purcell 2014). Finally, roots are often damaged due to road and house repair and construction.

Carbohydrate reserves of trees in storage pools (e.g., woody tissues) are an important mechanism by which trees have evolved to cope with disturbances, because they allow trees to maintain their metabolic activities and to start compensatory growth (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013). Allocation of photoassimilates to reserves normally compete with growth and other physiological processes such as defense (Chapin, Schulze & Mooney 1990). In general, carbohydrate reserves are comprised of non-structural carbohydrates (NSC) that are formed by low weight sugars and starch. Sugars are mobilized easily and used for short-term metabolism while starch is stored in a more recalcitrant form for long-term use during periods of severe stress (Chapin, Schulze & Mooney 1990; Dietze *et al.* 2013).

The concentration of NSC in tree tissues depends on the ability of organs to acquire plant-available resources (sink strength), and the distance between the carbon sources (either NSC in pools or carbohydrates synthesized by leaves) and carbon sinks (respiratory metabolism, storage of NSC, and tissue growth) (Lacointe 2000; Minchin & Lacointe 2005). Thus, disturbances that imply loss of tissue will affect the carbon allocation priorities for growth and reserves. This response will depend on the functional role of the organ/organs involved because these organs may function as carbon sources or carbon sinks (Li, Hoch & Körner 2002). For instance, defoliation causes a reduction in carbon sources and thus a decrease in the amount of photosynthates available for growth and reserves (Li, Hoch & Körner 2002; Eyles, Pinkard & Mohammed 2009; Quentin *et al.* 2011; Wiley *et al.* 2013; Atkinson *et al.* 2014; Jacquet *et al.* 2014; Deslauriers, Caron & Rossi 2015). After a defoliation event, the remaining leaves may increase their photosynthetic rates and their foliar nitrogen to compensate the supply of carbohydrates with little effect on overall growth and allocation (Reich *et al.* 1993; Lovelock, Posada & Winter 1999; Vanderklein & Reich 1999; Handa, Körner & Hättenschwiler 2005; Quentin *et al.* 2010; Quentin *et al.* 2011). Defoliation also requires the mobilization of NSC pools from branches, stems, and roots to maintain the metabolism and promote compensatory growth, which causes an additional reduction of the concentration of reserves that are stored in most of the tree organs.

Root pruning reduces the water supply to the leaves, which will reduce photosynthesis (carbon sources) (Vysotskaya *et al.* 2004) and also causes a reduction in total stored carbohydrates. Thus, root pruning should cause a reduction in total tree growth and a reallocation of resources belowground to quickly rebuild the root system (Ferree, Scurlock & Schmid 1999; Wajja-Musukwe *et al.* 2008; Dong *et al.* 2016). Also, roots are an important part of the tree woody biomass with a high

capacity to store carbohydrates (Landhäusser & Lieffers 2003). Thus, root pruning implies the loss of a great part of the tree's NSC storage pools.

The removal of the bark and cambium by stem damage has an impact on translocation via the phloem, but maintains water transport through the xylem. Removal of the phloem affects the mobilization and refilling of reserves between sources and sinks (Högberg *et al.* 2001; Moore 2013; Purcell 2014; Mei *et al.* 2015). Thus, the transport of reserves from roots to above-ground parts above the region ring-barked is reduced but so too the transport of photosynthates from the foliage to the root system (Moore 2013; Mei *et al.* 2015).

Unfortunately, in many cases more than one stress factor causes urban trees to become unhealthy and die (Calfapietra, Peñuelas & Niinemets 2015). The physiological response of trees to simultaneous stresses are generally unclear (Niinemets 2010). The interaction of several stress factors generates a unique response that may be more severe (negative interaction) or less severe (positive interaction) than the sum of their individual effects (Mittler 2006; Niinemets 2010). To date, relatively little is known about the effects of different individual stresses nor simultaneous stresses on both tree growth and the dynamics of NSC reserves in saplings and trees, because most of the studies about stress resistance have been carried out with seedlings subjected to a single stress (Niinemets 2010). To address this issue, we setup a manipulative experiment using three common North American urban tree species (*Fraxinus pennsylvanica*, *Celtis occidentalis*, and *Tilia cordata*). These trees were submitted to increasing levels of three common stresses under field conditions: (1) three levels of defoliation, (2) three levels of root pruning, and (3) two levels of stem damage. These stress treatments were applied individually and in combinations of two or three simultaneous stress factors.

We hypothesized that (*i*) tree growth (both in diameter and height) will be increasingly and negatively affected by increasing level of single and combined stresses inflicted on the trees; (*ii*) there would be mainly a negative effect on the level of NSC concentrations found in all four tree compartments (roots, stems, branches and leaves) measured in stressed trees that maintained their growth rate; (*iii*) there will be either a positive, negative or no effects of various combination of stresses depending on what eco-physiological mechanisms are being affected. For example, stem damage would limit the supply of reserves to either leaves (from roots) or roots (from new photosynthates), thus leading to a further reduction of reserves in these tissues. In contrast, there would be a positive interaction in NSC in treatments that involve defoliation and root pruning simultaneously, because defoliation may reduce the impact of water stress caused by root pruning. Thus, this should reduce the need to initiate compensatory growth to produce new roots and to exploit new water sources. And (*iv*) as a result of the competition for assimilates between tree growth and reserves, trees in single or combined stress treatments that resulted in higher carbohydrate concentrations would exhibit lower growth rates.

4.3 METHODS

4.3.1 Study site

The study was conducted in the municipal nursery of the city of Montreal, province of Quebec, Canada. The site lies at 45°30'' N, 73°33' W (about 35 m of elevation). The mean annual precipitation is 978 mm (215 mm snow and 763 mm rain). The mean annual temperature is 6.2 °C and the mean annual growing season temperature is 14.4 °C.

4.3.2 Study species

We studied three tree species that are among the most commonly planted trees in the city of Montreal: *Celtis occidentalis* Linnaeus (Common Hackberry; native), *Fraxinus pennsylvanica* Marsh. (Green ash; native), and *Tilia cordata* Mill. (little-leaf linden; introduced in America from Europe). The three tree species have different growth strategies and, thus, may present different responses of allocation to reserves and growth under stress (Table 4.1).

Table 4.1 Functional characteristics of the tree species studied.

Species/Trait	*Celtis occidentalis*	*Fraxinus pennsylvanica*	*Tilia cordata*
Foliar carbon (%)	41.03	46.47	47.00
Foliar nitrogen (%)	1.22	2.00	2.67
Foliar carbon/nitrogen	33.85	23.40	18.00
Specific leaf area ($mm^2\ mg^{-1}$)	17.27	15.17	18.60
Photosynthetic capacity ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	6.00	13.69	15.25
Wood density ($mg\ mm^{-3}$)	0.66	0.55	0.36
Growth rate	Moderate	Intermediate	Rapid
Shade tolerance*	Intermediate	Intermediate	Tolerant
Lifespan	Moderate	Short	Moderate

*Data from Niinemets and Valladares (2006)

4.3.3 Sapling treatments

Four-year-old trees of the aforementioned species with similar diameters and heights were selected in Montreal's municipal nursery. At the beginning of the study, trees were assigned randomly to gradients of three single stress treatments and various combinations of stresses (see details below). Treatments were first applied in July 2012 and repeated in July 2013, which corresponded to the month of maximum leaf area.

The stress treatments consisted of various gradients of defoliation, root reduction, and stem damage. The total number of samples in the experiment was 291 individuals (121 *C. occidentalis*, 91 *F. pennsylvanica,* and 79 *T. cordata*). The number of replicates per treatment was 6 in *C. occidentalis* and 4 in *F. pennsylvanica* and in *T. cordata*. The difference in the number of replicates was due to a lower availability of trees for the last two species. The experimental design was fully factorial including 3 levels of defoliation (0%, 33%, and 75%), 3 levels of root reduction (0%, 33%, and 75%), and 2 levels of stem damage (0% and 50%) with all possible combinations among these three treatments (Figure 4.1). The defoliation treatment consisted of removing leaves manually at the base of the petiole (Figure 4.1). Treatment intensity was defined as severe (75% defoliation); light (33% defoliation) and control (no defoliation). The root reduction treatment consisted of cutting a given percentage of the outermost part of a 30-cm radius of the root system with a tree spade machine (Figure 4.1). The machine consists of three or four blades that encircled the tree, dug into the ground independently, and cut the roots to a depth of 1.2 m. Treatment intensities were defined as severe (75 % root reduction), light (33% root reduction) and control (no root reduction). The stem damage treatment consisted of removing a 40-mm wide band at 30 cm above the ground using a barkblaster tree girdling tool until we had removed both the cambium and phloem connection (Figure 4.1).

Treatment intensities were 50% of the stem circumference length, and the control was no damage.

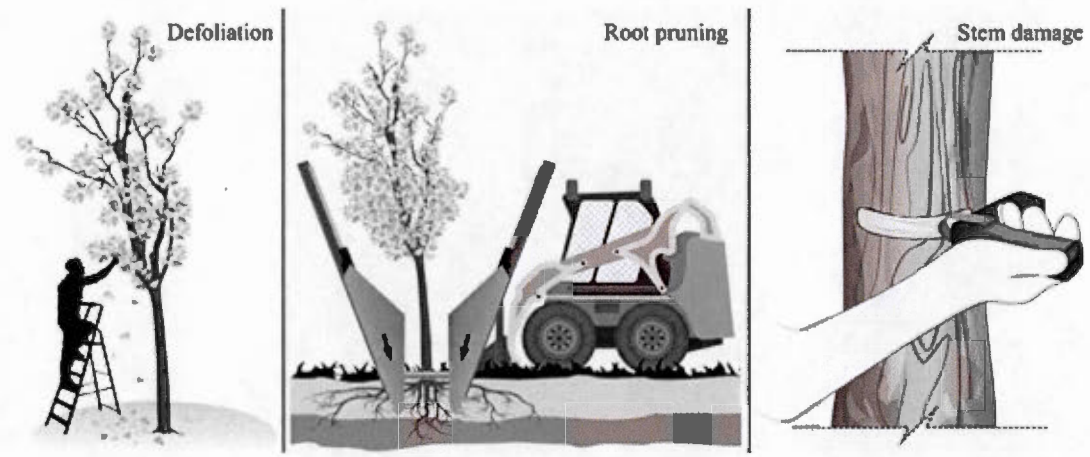

Figure 4.1 Schematic representation of the stress treatments applied to trees of F. pennsylvanica, C. occidentalis, and T. cordata.

Left: defoliation, center: root pruning. Right: stem damage.

4.3.4 Growth measurements

Trunk diameter and total tree height were measured bimonthly between July 2012 and November 2014. The diameter was measured at 40 cm above ground level to avoid branches that were actively growing. To increase accuracy, steel nails were inserted to mark the location of future measurements and the diameter was recorded on two sides of each individual. To allow comparisons between trees, growth measurements were normalized:

$$NG\ (t_i) = \left(G(t_i) - G(t_0)\right).(\overline{G(t_0)} / G(t_0)),$$

where *NG (t_i)* is the normalized growth at date *i*, *G(ti)* is the growth of the individual at date ti, *G(t0)* is the initial growth measure, and *(G(t0))* is the mean initial growth measure of all individuals.

4.3.5 Analysis of carbohydrate concentrations

The concentrations of NSC in roots, stems, and branches of all 291 saplings were determined in 2014 (at the beginning and at the end the growing season), one full season after the end of the last stress period to allow the trees to respond in terms of growth reallocation and reserve utilization. The concentrations of NSC in leaves were measured in the summer of 2014 only. In each individual, stem samples were taken with a 4.3-mm diameter increment borer at 130 cm above ground level. Top branches were obtained by cutting them with a tree trimmer. Root samples were taken with an increment borer from large surface roots within 10 cm from the base of the stem. Leaf samples were taken from the entire canopy and consisted of about 20 leaves per tree. Collected samples were placed in paper bags and refrigerated in the field. Within 8 h, they were microwaved in the lab to stop enzymatic activity (Popp *et al.* 1996), and then they were dried and grounded using a ball mill. Samples were analyzed for NSC concentration following Hoch, Popp and Körner (2002). Ground plant material was dissolved for 30 min in distilled water. Starch and sucrose were broken down into glucose and into glucose and fructose, respectively, with clarase (*Aspergillus oryzae*, Enzyme Solutions Pty Ltd, Crydon South, Victoria, Australia) incubation at 40°C for 15 h. Phosphoglucose-isomerase was added to the solution. The total amount of glucose, which corresponded to total NSC, was quantified photometrically in a microplate photometer at 340 nm (Thermo Fisher Scientific, Waltham, USA) after the conversion of glucose to gluconate-6-phosphate (hexokinase; Sigma-Aldrich, St. Louis, MO, USA). Subsequently, an aliquot of the original extract was treated with invertase and phosphoglucose-isomerase (both Sigma-Aldrich) to determine the amount of glucose, fructose, and sucrose with a glucose test (see above). Starch was calculated as NSC minus sugars (sugars = sucrose + fructose + glucose). Pure starch and glucose, fructose, and sucrose solutions were used as standards. Plant powder from peach leaves (Leco, St. Joseph, MI, USA) was included to control the

replicability of the extractions. NSC concentrations are reported here as percentage of dry matter.

4.3.6 Statistical analysis

Linear mixed-effect models were used to predict reserve concentrations (sugar, starch, and NSC) in the different tissues of the tree (leaves, roots, stems, and branches), and growth as a function of the three stress treatments. The models included the sampling blocks as a random effect. Models were evaluated using the R package "lme4" (Bates *et al.* 2014). The function *difflsmeans* in the "lmerTest" package (Kuznetsova, Brockhoff & Christensen 2016) was used as a post-hoc contrast to determine differences in the least square means of the models. The relationship between reserve concentrations and growth was assessed through the coefficient of determination (Pearson's r). All statistical analyses were conducted in R v. 3.02 (R Foundation for Statistical Computing, Vienna, Austria).

4.4 RESULTS

4.4.1 Effects of stress on tree growth

Root pruning alone caused a significant reduction in the diameter growth of the three species evaluated. Defoliation alone also caused a significant reduction of diameter growth but only in *C. occidentalis* and *T. cordata*. Stem damage alone caused a significant increase in diameter growth of *F. pennsylvanica* (Table 4.2, Figure 4.2). Defoliation caused a significant reduction in height growth of *C. occidentalis* and *F. pennsylvanica,* while root pruning only in *F. pennsylvanica* (Table 4.2, Figure 4.2). In most cases, a significant effect on tree growth was only achieved with severe stress (75% defoliation or 75% root pruning), the only exception was the effect of root pruning on diameter growth of *C. occidentalis* and *T. cordata* that was significant at 37% root pruning intensity. The interaction between stress treatments of defoliation and root pruning (DF:RP) reduced diameter growth on *C. occidentalis*. This effect is significant when there is an increase in defoliation from 37 to 75 % along with an increase in root pruning from 0 to 75%. The interaction between defoliation and stem damage (DF:SD) was also significant and reduced height growth on *F. pennsylvanica*, especially under light defoliation (37%) with stem damage (50%) (data not shown).

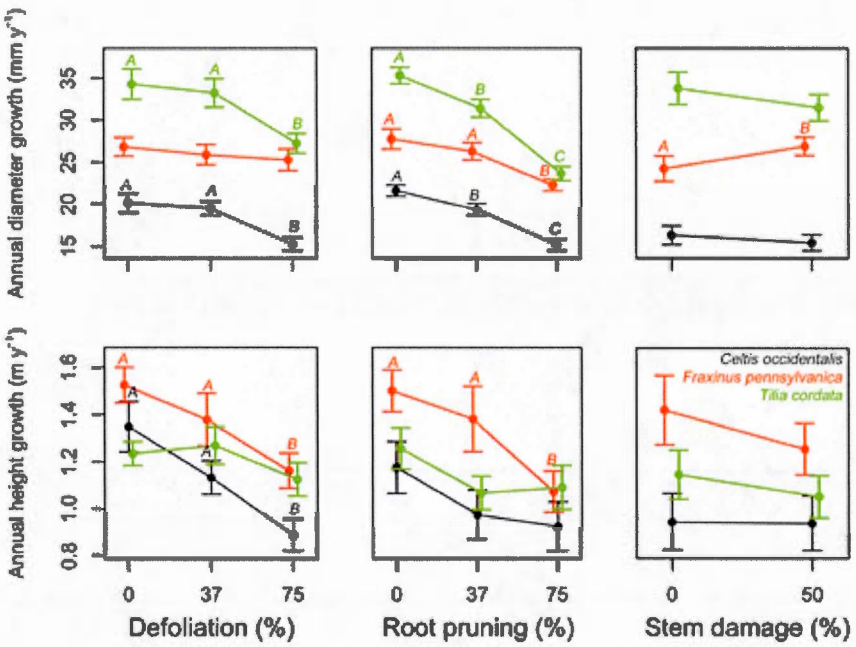

Figure 4.2 Single effects of a gradient of defoliation, root pruning, and stem damage on diameter and height growth of *Celtis occidentalis, Fraxinus pennsylvanica*, and *Tilia cordata*.

Error bars represent the standard error of the mean. Different letters represent significant mean differences between stress levels.

Table 4.2 Summary of linear mixed models effects showing the effects of defoliation (DF), root pruning (RP), and stem damage (SD) and their interacting effects on diameter and height at the end of the experiment of *Celtis occidentalis, Fraxinus pennsylvanica*, and *Tilia cordata* in the fall of 2014. Statistically significant models are shown in bold.

Growth		Diameter		Height	
		F stat	*p*-value	F stat	*p*-value
Celtis occidentalis	DF	15.43	**0.00**	8.71	**0.00**
	RP	34.24	**0.00**	1.79	0.17
	SD	0.12	0.73	0.05	0.83
	DF:RP	4.86	**0.00**	0.94	0.45
	DF:SD	0.53	0.59	0.13	0.88
	RP:SD	0.26	0.77	0.84	0.43
	DF:RP:SD	1.00	0.41	1.22	0.30
Fraxinus pennsylvanica	DF	1.16	0.32	6.39	**0.00**
	RP	12.93	**0.00**	5.06	**0.01**
	SD	7.03	**0.01**	0.09	0.76
	DF:RP	0.09	0.98	1.11	0.36
	DF:SD	0.69	0.50	4.11	**0.02**
	RP:SD	0.06	0.94	0.68	0.51
	DF:RP:SD	0.45	0.77	0.60	0.66
Tilia cordata	DF	17.60	**0.00**	1.54	0.23
	RP	59.06	**0.00**	1.48	0.24
	SD	0.55	0.46	0.05	0.81
	DF:RP	2.31	0.07	1.43	0.24
	DF:SD	0.15	0.86	0.43	0.66
	RP:SD	0.10	0.90	0.49	0.62
	DF:RP:SD	0.49	0.74	0.45	0.77

4.4.2 Effects of single stress treatments on NSC concentrations

At the moment of the first assessment (spring 2014), NSC concentrations in woody tissues varied between 0.4 -12.01% and at the second assessment (fall 2014) they varied between 1.1 - 11.7%, depending on the tissue, species, and treatments (Annex 2 and 3, respectively). Concentrations of NSC in leaves in summer varied between 3.3 -12.7%; the highest were in *C. occidentalis* and the lowest in *F. pennsylvanica* (Annex 4).

Not all the single stress treatments showed a significant effect on the concentrations of reserves in both periods evaluated (spring and fall. Table 4.3, and Figure 4.3). Overall, the significant effects in both periods showed that a severe increase in single stress (by defoliation, root pruning or stem damage) increased significantly reserve concentrations in stems and roots, but reduced concentrations in branches (Figure 4.3). Single treatments of root pruning and stem damage decreased significantly the NSC concentrations of leaves of *C. occidentalis* only (Table 4.3, Annex 4).

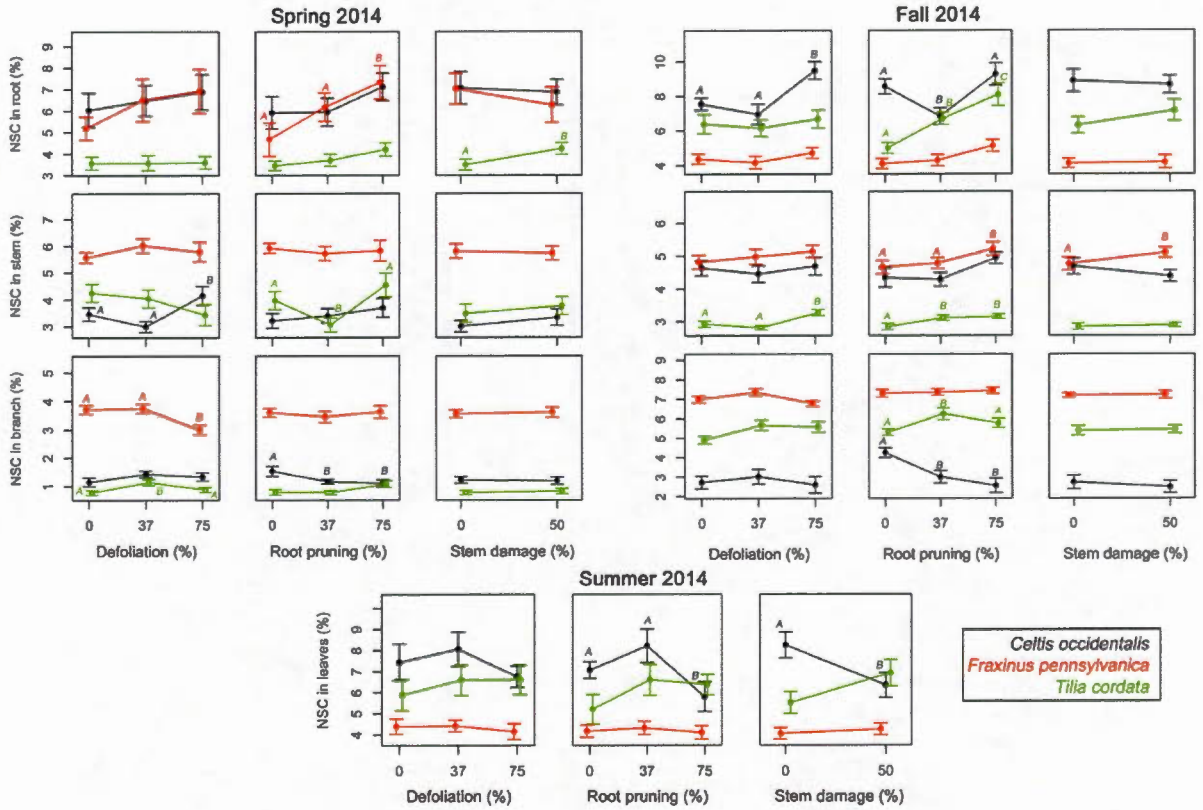

Figure 4.3 Single effects of a gradient of defoliation, root pruning, and stem damage on non-structural carbohydrates (NSC) concentrations tissues of *Celtis occidentalis*, *Fraxinus pennsylvanica,* and *Tilia cordata.*

Error bars represent the standard error of the mean. Different letters represent significant mean differences between stress levels..

Table 4.3 Summary of linear mixed models results showing the effects of defoliation (DF), root pruning (RP), and stem damage (SD) and their interacting effects on NSC concentrations of *Celtis occidentalis, Fraxinus pennsylvanica*, and *Tilia cordata* in the spring and fall of 2014. Statistically significant models are shown in bold.

Tissue		Root (spring 2014)		Root (fall 2014)		Stem (spring 2014)		Stem (fall 2014)		Branch (spring 2014)		Branch (fall 2014)		Leaves (summer 2014)	
		F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value
Celtis occidentalis	DF	0.28	0.76	8.92	**0.00**	5.28	**0.01**	0.29	0.74	1.22	0.31	0.39	0.68	1.00	0.38
	RP	0.97	0.39	6.40	**0.00**	0.61	0.55	2.79	0.08	3.57	**0.04**	6.73	**0.00**	4.09	**0.03**
	SD	0.04	0.85	0.18	0.67	0.90	0.35	1.14	0.29	0.13	0.72	0.32	0.58	6.19	**0.02**
	DF:RP	0.47	0.76	0.77	0.55	0.18	0.95	1.20	0.33	0.64	0.63	0.53	0.71	0.44	0.78
	DF:SD	0.32	0.73	1.22	0.31	3.58	**0.04**	0.04	0.96	1.76	0.19	1.18	0.32	0.10	0.90
	RP:SD	0.58	0.56	4.01	**0.02**	1.69	0.20	0.09	0.91	1.14	0.33	0.57	0.57	3.27	**0.05**
	DF:RP:SD	2.28	0.08	0.82	0.52	0.32	0.86	0.36	0.84	2.49	0.06	0.87	0.49	1.97	0.12
Fraxinus pennsylvanica	DF	1.55	0.23	1.01	0.37	0.52	0.60	1.90	0.16	6.25	**0.00**	2.40	0.10	0.41	0.66
	RP	3.90	**0.03**	2.95	0.07	0.08	0.93	6.27	**0.00**	0.57	0.57	0.15	0.86	0.27	0.76
	SD	0.89	0.35	2.00	0.66	0.03	0.85	4.49	**0.04**	0.00	0.95	0.02	0.88	0.10	0.75
	DF:RP	1.68	0.18	0.83	0.51	0.28	0.89	2.22	0.08	0.27	0.89	0.96	0.44	1.06	0.39
	DF:SD	5.11	**0.01**	0.73	0.49	0.63	0.54	0.28	0.76	2.55	0.09	0.29	0.75	0.22	0.81
	RP:SD	0.36	0.70	1.23	0.30	1.77	0.18	0.08	0.93	0.95	0.40	0.35	0.71	5.80	**0.01**
	DF:RP:SD	0.34	0.85	1.02	0.41	0.64	0.63	0.98	0.43	0.22	0.92	0.32	0.86	1.24	0.31
Tilia cordata	DF	0.00	0.99	0.38	0.69	1.60	0.22	12.48	**0.00**	4.18	**0.02**	2.99	0.06	0.35	0.71
	RP	2.24	0.12	11.20	**0.00**	4.47	**0.02**	6.84	**0.00**	3.05	0.06	3.73	**0.03**	1.35	0.27
	SD	4.41	**0.04**	1.69	0.20	0.41	0.53	0.38	0.54	0.18	0.67	0.04	0.85	2.92	0.10
	DF:RP	1.26	0.30	0.42	0.79	0.50	0.74	0.88	0.48	1.31	0.29	0.87	0.49	0.44	0.78
	DF:SD	0.99	0.38	0.32	0.73	1.45	0.25	0.73	0.49	0.36	0.70	0.05	0.95	0.99	0.38
	RP:SD	0.67	0.52	0.99	0.38	0.38	0.69	0.23	0.80	0.58	0.56	0.57	0.57	0.63	0.54
	DF:RP:S	0.90	0.47	0.92	0.46	1.55	0.21	1.16	0.35	0.77	0.56	0.36	0.84	1.48	0.23

4.4.3 Effects of combined stress treatments on NSC concentrations

In both evaluation periods, the combined stress treatments that showed significant interactions on reserve concentrations were: (1) for *Celtis occidentalis* in the root between root pruning and stem damage in fall 2014, in the stem between defoliation and stem damage in spring 2014 and in the leaves between root pruning and stem damage; and (2) for *Fraxinus pennsylvanica* in the root between defoliation and stem damage in spring 2014 and the leaves between root pruning and stem damage (Table 4.3; Figure 4.4). No significant interactions among any of the three stress treatments were found for *Tilia cordata*. As seen in Fig. 4, in spring 2014, a light defoliation (37%) along with an increase in stem damage decreased reserve concentrations in roots of *F. pennsylvanica*. However, severe defoliation (75%) with an increase in stem damage increased reserve concentrations in roots of *F. pennsylvanica* (Figure 4.4).

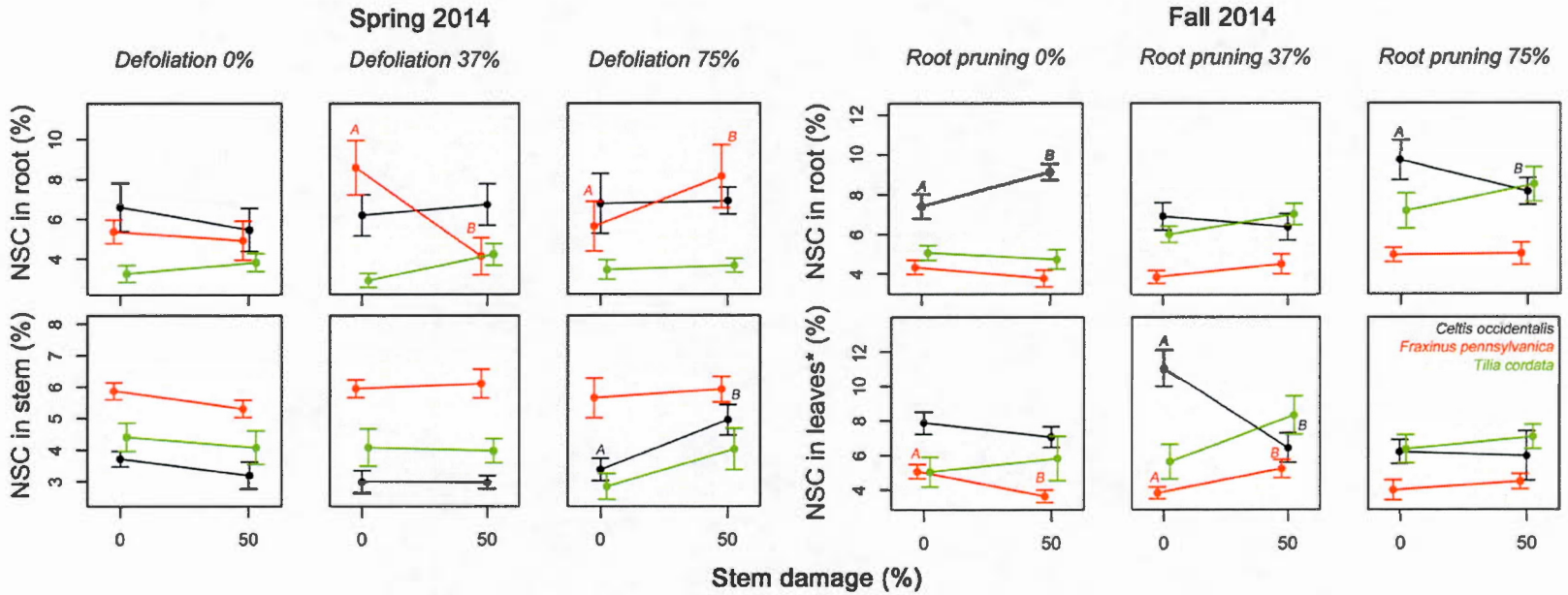

Figure 4.4 Significant interacting effects of defoliation, root pruning, and stem damage on non-structural carbohydrates (NSC) concentrations in tissues of *Celtis occidentalis, Fraxinus pennsylvanica*, and *Tilia cordata.*

Error bars represent the standard error of the mean. Different letters represent significant mean differences between stress levels. *NSC evaluation in leaves was performed in summer 2014.

4.4.4 Relationship between NSC concentrations and tree growth

We found in several tissues of the three species that the trees with higher NSC concentrations were those with lower diametric increment (Figure 4.5). Specifically, we found significant negative correlations between diameter increment and NSC concentrations in roots and stems of *C. occidentalis*, roots of *F. pennsylvanica* and roots, stems and branches of *T. cordata*. On the contrary, we did not find any significant correlation between height increment and NSC concentrations in tree tissues of the three species in both evaluated periods (data not shown).

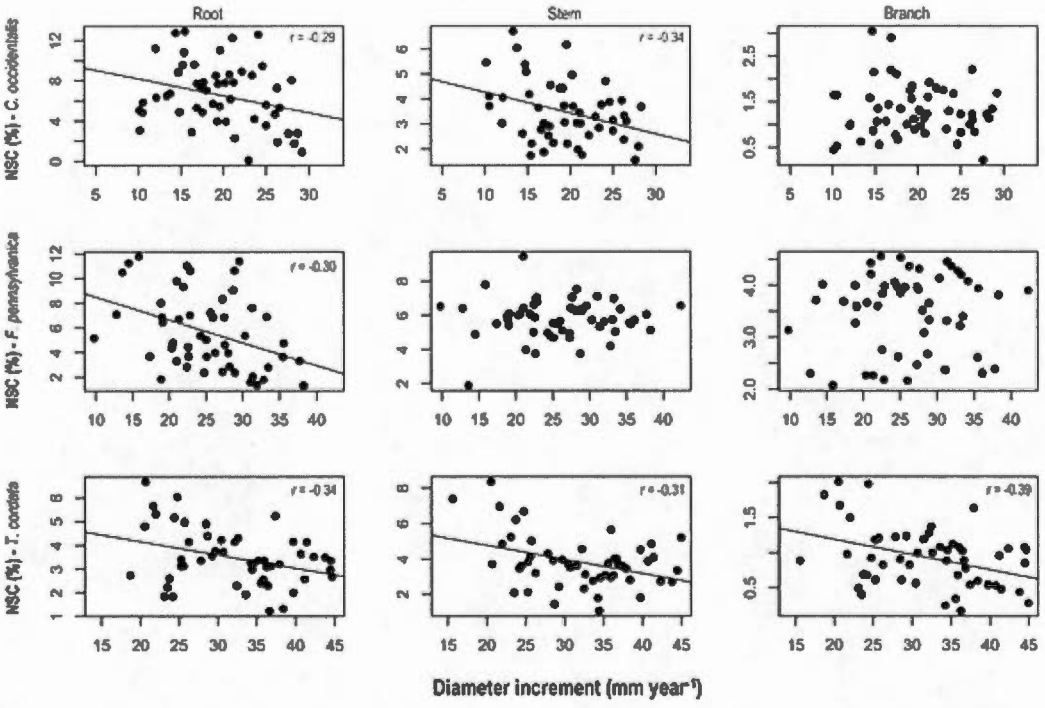

Figure 4.5 Relationships between non-structural carbohydrates concentrations in spring 2014 and diameter increment in *Celtis occidentalis, Fraxinus pennsylvanica,* and *Tilia cordata.*

Model lines represent significant relationships between non-structural carbohydrates concentrations and diameter increment.

4.5 DISCUSSION

Our results describe the single impact of different levels of defoliation, root pruning, and stem damage and their combined effects on tree growth and carbohydrate reserves for three common urban tree species. We found that tree diameter and height growth decreased in relation to the levels of stress applied to the trees (with the exception of stem damage on *F. pennsylvanica* where diameter growth increased). The effects of the different stress treatments on NSC in the different parts of the tree were much more varied among the three tree species and we found some significant interactions among the different stress treatments that make any generalizable interpretation difficult. Overall, the effects of single treatments caused an increase in the levels of reserves in stem and roots, and a decrease of the levels of reserves in branches. On the contrary, the effects of the combined stress treatments on reserve were very variable depending on the species and the tree part evaluated. However, we found globally a decline in tree growth for all three species with an increase in the overall NSC stored in the root and stem and in the branch for *T. Cordata*.

4.5.1 Effects of stress treatments on tree growth

As hypothesized, tree growth (both in diameter and height) was negatively affected by the increasing level of stresses inflicted on the trees (Table 4.2, Figure 4.2). Overall, annual growth in diameter and height was reduced up to 47% and 55%, respectively. Although the growth responses to stress treatments are complex and depend on the species and type of stress applied, our results are similar to the values reported for defoliation and root pruning in other studies (≈35-50%, Quentin *et al.* 2011; Jacquet, Orazio & Jactel 2012; Wiley *et al.* 2013; Dong *et al.* 2016). This reduction in growth was expected due to defoliation, which limits carbon uptake, and due to root pruning, which reduces water and nutrients uptake. Finally, although we

expected stem damage to decrease tree growth by disrupting the transport of photosynthates to the roots and affecting their physiological functions (Regier *et al.* 2010; Mei *et al.* 2015), we found in one instance a significant increase in tree diameter for *F. pennsylvanica.* This increase in tree diameter may have been due to accumulation of carbohydrates above the wound zone as none is able to get the root system (Moore 2013).

4.5.2 Effects of stress treatments on reserve concentrations

We hypothesized that there would be a negative effect on the level of reserve concentrations in tree tissues in several stress treatments where trees are able to maintain growth rate. Our results do not fully support our hypothesis as we found that most of the single and combined stresses caused an increase in the levels of reserves in stem and roots and a decrease in tree growth rate, although we found a decrease in the levels of reserves in branches (Table 4.3 and, Figure 4.3). Several papers have reported a reduction of carbohydrate reserves just weeks or few months after stress conditions caused by defoliation (Palacio *et al.* 2012; Wiley *et al.* 2013; Atkinson *et al.* 2014), pruning (Chesney & Vasquez 2007), and stem girdling, especially from the tissues that mobilized reserves to maintain physiological activities (Mei *et al.* 2015). In this paper, we did our first assessment of carbohydrate reserve concentrations about nine months after the last stress treatments were applied to allow time for the trees to respond in terms of growth reallocation. Except for the concentrations of carbohydrates in branches after some stress treatments, our results of carbohydrate concentrations in stem and roots are consistent with the fast recovery and increase of reserves after severe episodes of stress regardless of the species or source of stress.

An increase of carbohydrate concentrations to the pre-stress levels in the main stem and roots after carbon-limiting conditions induced by stress treatments may indicate

that these tissues are a more secure place to store carbohydrates, ensuring that resources are available for resprouting or refoliating after future stress episodes (Gibon *et al.* 2009; Wiley *et al.* 2013). This idea is supported by the lower sugar to starch ratio found in these tissues (data not shown), indicating that trees might prioritize the accumulation of the more stable starch for long-term use during periods of severe stress (Dietze *et al.* 2013). On the contrary, there was a reduction in reserve concentrations in branches. Across the species the mean sugar to starch ratio was higher in branches that in other woody tissues (data not show). This suggests a higher mobility of sugars from tissues to maintain metabolic activity and compensatory growth of new foliar resources after stress (Landhäusser & Lieffers 2003; Landhäusser 2011), and thus the reduction in concentrations after stress.

The increase in carbohydrate reserve concentrations in stems and roots after the stress treatments may have been reached through compensatory mechanisms, such as increasing nitrogen concentrations and photosynthetic rates of the remaining foliage after defoliation (Pinkard & Beadle 1998; Vanderklein & Reich 1999; Eyles, Pinkard & Mohammed 2009). Carbohydrate concentrations in leaves can provide insights about these compensatory responses in these trees, because it is expected that an increase in nitrogen concentrations and photosynthetic rates leads to an increase in reserves in leaves (Li *et al.* 2016). Nevertheless, unlike woody tissues, few stress treatments had a significant effect on reserve concentrations in leaves, and those that had a significant effect caused a reduction on the carbohydrate reserves, which suggests no evidence of photosynthetic up-regulation and compensatory responses (Annex 7).

We hypothesized that there were either a positive, negative or no effects of the combination of stresses depending on the mechanisms affected. We expected a positive interaction in reserve concentrations in treatments that involved defoliation

and root pruning simultaneously compared to single stress treatments. Yet, none of these interactions was significant (Table 3). On the contrary, we believed that a combination of tissue loss (by either defoliation or root pruning) and stem damage would lead to a negative interaction (reduction of reserve concentrations) because stem damage limits the supply of reserves to either to leaves (from roots) or to roots (from new photosynthates). Overall, we found several significant interactions between tissue loss and stem damage but with different patterns that make a generalizable interpretation difficult. We found that severe defoliation (75%) with stem damage (50%) increased reserve concentrations in roots and stems of *C. occidentalis* and *F. pennsylvanica* (Figure 4.4). As we suggested before, this may indicate the mobilization of reserves to more secure tissues under severe stress conditions (Gibon *et al.* 2009; Wiley *et al.* 2013). However, light defoliation (37%) with stem damage (50%) reduced reserve concentrations in roots. Severe root pruning (75%) and stem damage (50%) also caused a reduction in reserve concentrations in roots of *C. occidentalis,* which may indicate that stem damage is limiting the supply of reserves from leaves to roots and thus, roots are expending their accumulating reserves in metabolism and/or increasing root production to exploit new available soil nutrients and water resources (Mei *et al.* 2015). Light root pruning (37%) and stem damage (50%) caused a contrasting effect in reserve concentrations of leaves of *C. occidentalis* and *F. pennsylvanica* (Figure 4.4). In *C. occidentalis* concentrations decreased while in *F. pennsylvanica* concentrations increased. A decrease in reserve concentrations after stress treatments in *C. occidentalis* may indicate the lack of mechanisms such as compensatory photosynthesis to recover carbon supply (McNaughton 1983). A reduction in the carbon supply by photosynthesis may lead to fast depletion of the reserve pools and thus increasing the stress effect on the trees (Niinemets 2010).

4.5.3 Relationship between carbohydrate reserves and tree growth

As we hypothesized, we found a negative relationship between NSC concentrations and diameter increment (Figure 4.5). Nevertheless, this relationship was not significant in all storage tissues for all three species. Our results suggest that following the disturbance of some parts of the trees, trees may mobilize accumulated stored NSC over the short term to repair the damage and increase growth, but over the medium to long-term the strategy seems to replenish as quickly as possible the reserve pool at the detriment of tree growth. Increasing reserves under the conditions of lower carbon uptake imposed by the stress treatments is consistent with previous studies that suggested that allocation of carbon to reserves is an active process that does not depend on the balance between carbon supply and demand for growth and metabolism; that is, trees regulate the levels of reserves at the expense of growth (Chapin, Schulze & Mooney 1990; Silpi *et al.* 2007; Sala, Woodruff & Meinzer 2012; Wiley & Helliker 2012). Such behavior in carbohydrate reserves suggests that trees adjust their levels of reserves to meet the new metabolic demand (Silpi *et al.* 2007), because survival under the stress conditions may require higher availability of carbon for maintaining physiological functions, such as metabolism, hydraulic integrity and osmotic exchange of the soluble sugars, instead of maintaining growth (Sala, Woodruff & Meinzer 2012; Wiley & Helliker 2012).

The negative relationship between NSC concentrations and diameter increment was significant in the three woody tissues of *T. cordata* and persistent in roots of the three tree species. This may indicate that trees of *T. cordata* showed higher response to stress than the other two species. Although *T. cordata* is a shade tolerant species, it presents strategies of fast growing species such as high foliar nitrogen, photosynthetic capacity, and lower wood density (Table 4.1). This may indicate lower allocation of carbon to defense traits and thus higher dependence of reserves

than the other species to maintain a positive carbon balance. This results support the idea that fast growing species respond with higher flexibility than slow growing species under stress conditions (Atkinson *et al.* 2014).

4.6 CONCLUSIONS

This study examined the single and combined effects of three common urban stresses (defoliation, root pruning, and stem damage) on the growth and NSC reserve accumulation in four-year-old trees under field conditions. Our results showed a consistent inverse relationship between diameter growth and total NSC reserve in all three tree species, indicating that there is an active process of allocating reserves at the expense of tree growth. Globally, trees tended to accumulate NSC in roots and stems (but not in branches) 9 to 12 months following various combination of stresses, but we found some significant interactions between the three types of stresses applied indicating that some combination of stresses could modify the general trend found when single stresses are applied. These results are useful for predicting plant performance and survivorship under different urban stress conditions.

4.7 SUPPORTING INFORMATION

4.7.1 Annex A

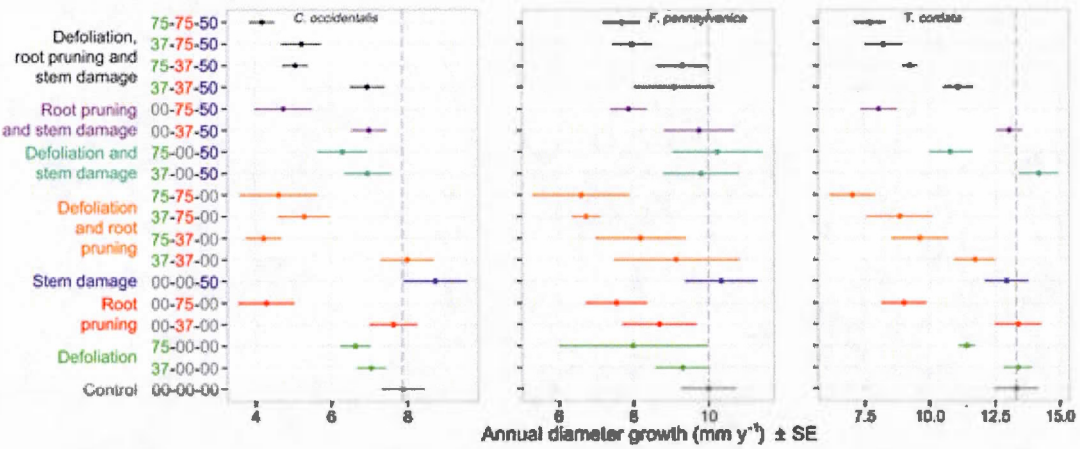

Figure 4.6A Means and standard errors of annual diameter growth in fall 2014 after both single stress treatments of defoliation (DF, green numbers, on the left), root pruning (RP, red numbers, in the center), and stem damage (SD, blue numbers, on the right) and their combined effects in *Celtis occidentalis, Fraxinus pennsylvanica*, and *Tilia cordata.* The vertical dashed line corresponds to the average annual diameter growth of the control.

4.7.2 Annex B

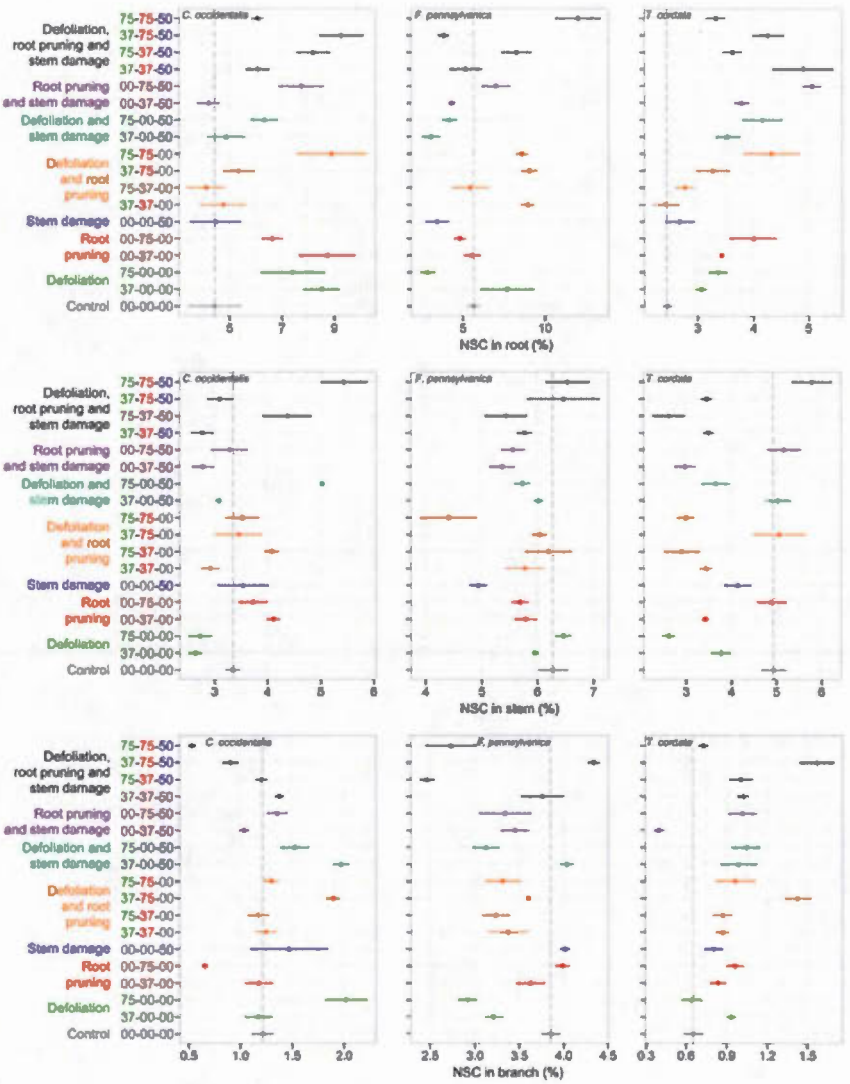

Figure 4.7B Means and standard errors of non-structural carbohydrates concentrations in spring 2014 after both single stress treatments of defoliation (DF, green numbers, on the left), root pruning (RP, red numbers, in the center), and stem damage (SD, blue numbers, on the right) and their combined effects in *Celtis occidentalis, Fraxinus pennsylvanica,* and *Tilia cordata.*

4.7.3 Annex C

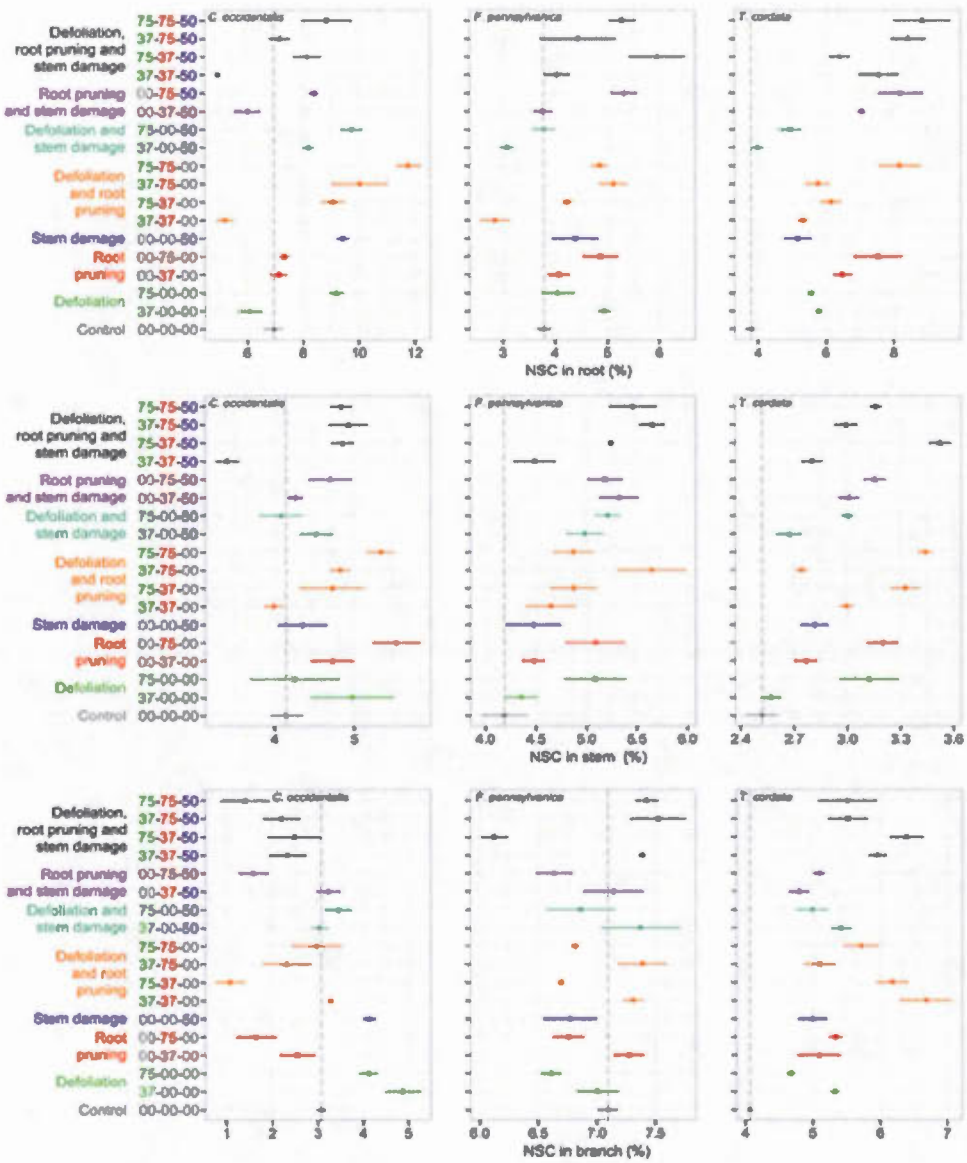

Figure 4.8C Means and standard errors of non-structural carbohydrates concentrations in fall 2014 after both single stress treatments of defoliation (DF, green numbers, on the left), root pruning (RP, red numbers, in the center), and

stem damage (SD, blue numbers, on the right) and their combined effects in *Celtis occidentalis, Fraxinus pennsylvanica,* and *Tilia cordata.*

4.7.4 Annex D

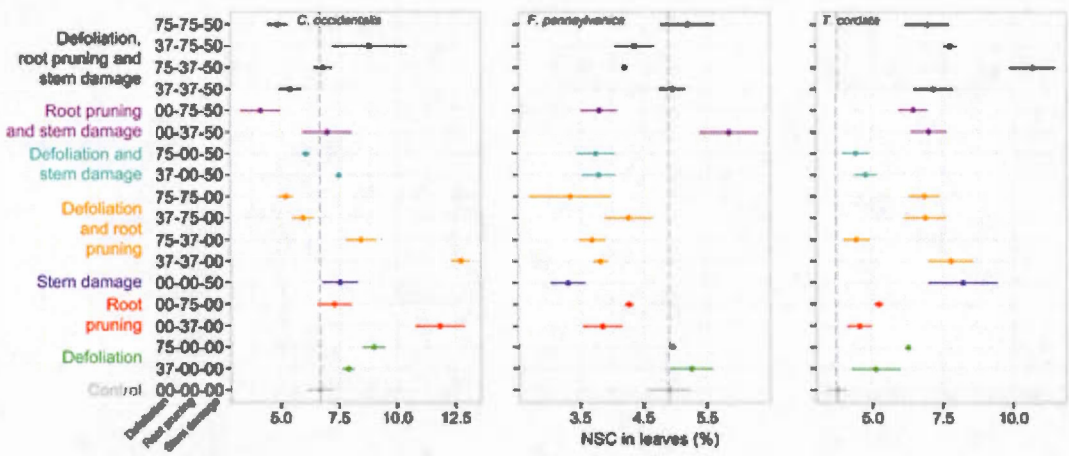

Figure 4.9D Means and standard errors of NSC concentrations in leaves in summer 2014 after both single stress treatments of defoliation (DF), root pruning (RP), and stem damage (SD) and their combined effects in *Celtis occidentalis, Fraxinus pennsylvanica* and *Tilia cordata.*

4.7.5 Annex E

Table 4.4E Summary of linear mixed models results showing the single effects of defoliation (DF), root pruning (RP), and stem damage (SD) and their interacting effects on concentrations of sugar and starch of *Celtis occidentalis, Fraxinus pennsylvanica,* and *Tilia cordata.* in spring 2014. Statistically significant models are shown in bold.

Tissue		Root				Stem				Branch			
Carbohydrate		Sugar		Starch		Sugar		Starch		Sugar		Starch	
		F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value
Celtis occidentalis	DF	2.01	0.15	0.46	0.63	1.51	0.24	3.71	**0.04**	0.62	0.54	1.97	1.16
	RP	7.21	**0.00**	0.20	0.82	0.50	0.61	0.86	0.43	0.68	0.51	3.56	**0.04**
	SD	1.48	0.23	0.00	0.93	0.16	0.69	0.72	0.40	0.66	0.42	0.02	0.90
	DF:RP	1.72	0.17	0.59	0.67	0.89	0.48	0.14	0.97	1.18	0.33	2.18	0.09
	DF:SD	0.37	0.70	0.27	0.77	1.79	0.18	5.12	**0.01**	0.59	0.56	1.18	0.32
	RP:SD	0.61	0.55	0.86	0.43	0.74	0.48	1.09	0.35	1.85	0.17	0.30	0.74
	DF:RP:SD	0.73	0.58	2.76	**0.04**	0.62	0.65	0.27	0.90	0.97	0.44	2.10	0.10
Fraxinus pennsylvanica	DF	0.89	0.42	1.39	0.26	0.69	0.51	1.05	0.36	3.35	**0.05**	0.06	0.94
	RP	0.16	0.85	3.70	**0.04**	3.34	**0.05**	0.73	0.49	0.39	0.68	0.49	0.62
	SD	4.85	**0.03**	0.50	0.48	0.07	0.80	0.08	0.77	0.00	0.93	0.00	0.97
	DF:RP	1.20	0.33	1.46	0.23	2.05	0.11	0.81	0.52	0.74	0.57	0.22	0.92
	DF:SD	4.67	**0.02**	4.18	**0.02**	0.84	0.44	1.21	0.31	1.40	0.26	0.37	0.69
	RP:SD	0.88	0.42	0.28	0.75	2.23	0.12	1.04	0.36	0.79	0.46	2.08	0.14
	DF:RP:SD	1.45	0.24	0.37	0.83	1.21	0.32	0.40	0.81	0.71	0.59	0.49	0.74
Tilia cordata	DF	0.77	0.47	0.55	0.58	0.16	0.86	1.33	0.28	4.34	**0.02**	2.31	0.11
	RP	1.52	0.23	0.82	0.45	1.75	0.19	2.27	0.12	1.29	0.29	4.06	**0.03**
	SD	0.00	0.99	7.99	**0.01**	1.77	0.19	0.12	0.74	1.02	0.32	0.95	0.33
	DF:RP	1.02	0.41	1.14	0.35	0.50	0.74	1.16	0.34	1.84	0.14	0.92	0.46
	DF:SD	0.47	0.63	0.66	0.52	0.17	0.84	1.37	0.27	1.46	0.25	4.06	**0.03**
	RP:SD	0.59	0.56	0.22	0.80	0.45	0.64	0.52	0.60	0.73	0.49	0.12	0.88
	DF:RP:SD	0.64	0.64	1.50	0.22	1.49	0.23	0.93	0.46	0.81	0.53	1.48	0.23

4.7.6 Annex F

Table 4.5F Summary of linear mixed models results showing the single effects of defoliation (DF), root pruning (RP), and stem damage (SD) and their interacting effects on concentrations of sugar and starch of *Celtis occidentalis*, *Fraxinus pennsylvanica,* and *Tilia cordata.* in fall 2014. Statistically significant models are shown in bold.

Tissue		Root				Stem				Branch			
Carbohydrate		Sugar		Starch		Sugar		Starch		Sugar		Starch	
		F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value	F stat	*p*-value
Celtis occidentalis	DF	3.00	0.06	8.48	**0.00**	0.66	0.52	0.09	0.91	0.53	0.59	2.02	0.15
	RP	4.48	**0.02**	5.32	**0.01**	6.04	**0.01**	0.49	0.62	7.61	**0.00**	0.88	0.42
	SD	0.59	0.44	0.04	0.85	0.60	0.44	0.59	0.45	0.37	0.54	0.10	0.75
	DF:RP	1.83	0.15	0.94	0.45	1.49	0.23	0.39	0.81	0.57	0.69	0.44	0.78
	DF:SD	0.57	0.57	1.06	0.36	0.61	0.55	0.10	0.90	1.27	0.29	0.22	0.80
	RP:SD	3.14	0.06	3.31	**0.05**	0.21	0.81	0.01	0.99	0.50	0.61	0.37	0.69
	DF:RP:SD	0.46	0.77	1.36	0.26	0.11	0.98	0.41	0.80	0.74	0.57	1.12	0.36
Fraxinus pennsylvanica	DF	0.22	0.81	3.78	**0.03**	0.17	0.84	0.38	0.68	2.05	0.15	1.90	0.16
	RP	1.07	0.36	3.75	**0.03**	7.38	**0.00**	0.42	0.66	0.50	0.61	0.74	0.48
	SD	0.03	0.87	0.78	0.38	1.21	0.27	0.51	0.47	0.13	0.73	0.22	0.64
	DF:RP	0.41	0.80	1.41	0.25	0.57	0.68	1.69	0.17	1.21	0.33	0.84	0.51
	DF:SD	0.79	0.46	0.46	0.63	0.12	0.89	0.53	0.59	0.51	0.61	0.86	0.43
	RP:SD	1.62	0.21	0.78	0.46	0.42	0.66	0.36	0.70	1.00	0.38	1.21	0.31
	DF:RP:SD	1.71	0.17	0.57	0.68	1.44	0.24	0.31	0.87	0.52	0.72	0.92	0.46
Tilia cordata	DF	0.13	0.88	1.65	0.20	10.62	**0.00**	2.24	0.12	2.99	0.06	1.84	17.00
	RP	32.68	**0.00**	2.57	0.09	13.85	**0.00**	3.94	**0.03**	3.73	**0.03**	2.25	0.12
	SD	2.83	0.10	0.17	0.68	1.81	0.19	1.83	0.19	0.04	0.85	0.01	0.76
	DF:RP	0.54	0.70	0.35	0.84	1.41	0.25	2.39	0.07	0.87	0.49	0.74	0.57
	DF:SD	0.31	0.74	0.38	0.69	1.36	0.27	0.21	0.81	0.05	0.95	0.02	0.97
	RP:SD	1.84	0.17	0.49	0.62	0.15	0.87	0.27	0.76	0.57	0.57	0.85	0.44
	DF:RP:SD	1.02	0.41	0.64	0.64	1.24	0.31	1.40	0.26	0.36	0.84	0.45	0.77

4.7.7 Annex G

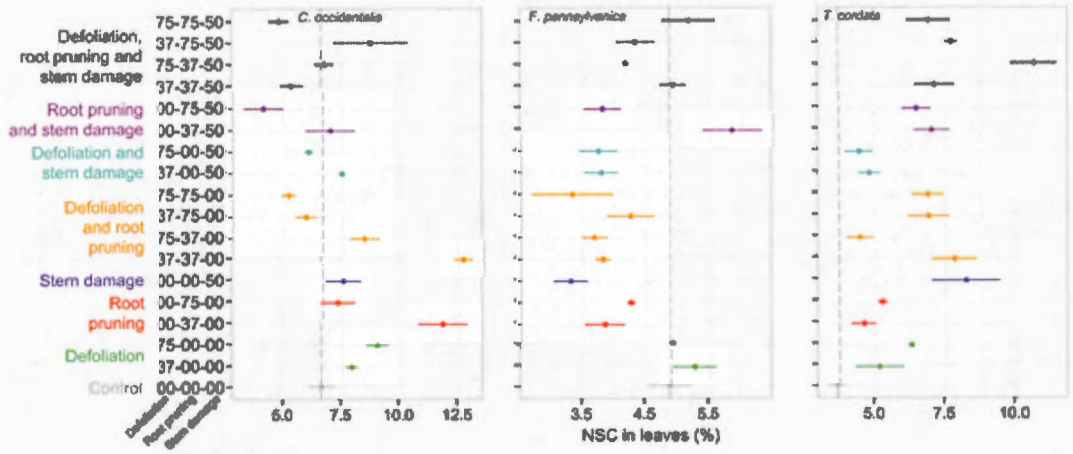

Figure 4.10G Means and standard errors of NSC concentrations in leaves in summer 2014 after both single stress treatments of defoliation (DF), root pruning (RP), and stem damage (SD) and their combined effects in *Celtis occidentalis*, *Fraxinus pennsylvanica* and *Tilia cordata*.

4.8 REFERENCES

Atkinson, R. R. L., M. M. Burrell, K. E. Rose, C. P. Osborne, and M. Rees. 2014. The dynamics of recovery and growth: how defoliation affects stored resources. Proceedings of the Royal Society B **281**:20133355.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7.

Calfapietra, C., J. Peñuelas, and Ü. Niinemets. 2015. Urban plant physiology: adaptation-mitigation strategies under permanent stress. Trends in Plant Science **20**:72-75.

Chapin, F. S., E. D. Schulze, and H. A. Mooney. 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics **21**:423-447.

Chesney, P., and N. Vasquez. 2007. Dynamics of non-structural carbohydrate reserves in pruned Erythrina poeppigiana and Gliricidia sepium trees. Agroforestry Systems **69**:89–105.

Deslauriers, A., L. Caron, and S. Rossi. 2015. Carbon Allocation during Defoliation: Testing a Defense-Growth Trade-off in Balsam Fir. Frontiers in Plant Science **6**:338.

Dietze, M. C., A. Sala, M. S. Carbone, C. I. Czimczik, J. A. Mantooth, A. D. Richardson, and R. Vargas. 2013. Nonstructural Carbon in Woody Plants. Annual Review of Plant Biology **65**:2.1–2.21.

Dong, T., B. Duan, S. Zhang, H. Korpelainen, Ü. Niinemets, and C. Li. 2016. Growth, biomass allocation and photosynthetic responses are related to intensity of root severance and soil moisture conditions in the plantation tree Cunninghamia lanceolata. Tree Physiology.

Eyles, A., E. A. Pinkard, and C. Mohammed. 2009. Shifts in biomass and resource allocation patterns following defoliation in Eucalyptus globulus growing with varying water and nutrient supplies. Tree Physiology **29**:753–764.

Ferree, D. C., D. M. Scurlock, and J. C. Schmid. 1999. Root pruning reduces photosynthesis, transpiration, growth, and fruiting of container-grown French-American hybrid grapevines. HortScience **34**:1064–1067.

Gibon, Y., E. Pyl, R. Sulpice, J. Lunn, M. Hohne, M. Gunther, and M. Stitt. 2009. Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when Arabidopsis is grown in very short photoperiods. Plant Cell and Environment **32**:859–874.

Handa, T., C. Körner, and S. Hättenschwiler. 2005. A test of the treeline carbon limitation hypothesis by in situ CO2 enrichment and defoliation. Ecology **86**:1288-1300.

Hoch, G., M. Popp, and C. Körner. 2002. Altitudinal increase of mobile carbon pools in Pinus cembra suggests sink limitation of growth at the Swiss treeline. Oikos **98**:361–374.

Högberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Ekblad, M. N. Högberg, G. Nyberg, M. Ottosson-Löfvenius, and D. J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature **411**:789–792.

Jacquet, J. S., A. Bosc, A. O'Grady, and H. Jactel. 2014. Combined effects of defoliation and water stress on pine growth and non-structural carbohydrates. Tree Physiology **00**:1–10.

Jacquet, J. S., C. Orazio, and H. Jactel. 2012. Defoliation by processionary moth significantly reduces tree growth: a quantitative review. Annals of Forest Science **69**:857–866.

Konijnendijk, C. C., K. Nilsson, T. B. Randrup, and J. Schipperijn. 2005. Urban Forests and Trees. Springer-Verlag, Berlin.

Konijnendijk, C. C., and T. B. Randrup. 2004. Urban forestry. Pages 471-478 *in* J. Burley, J. Evans, and J. Youngquist, editors. Encyclopedia of Forest Sciences. Academic Press, Amsterdam.

Kuznetsova, A., P. B. Brockhoff, and R. H. B. l. Christensen. 2016. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-30.

Lacointe, A. 2000. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Annals of Forest Science **57**:521–533.

Landhäusser, S. M. 2011. Aspen shoots are carbon autonomous during bud break. Trees **25**:531–536.

Landhäusser, S. M., and V. J. Lieffers. 2003. Seasonal changes in carbohydrate reserves in mature northern Populus tremuloides clones. Trees **17**:471–476.

Li, M. H., G. Hoch, and C. Körner. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. Trees-Structure and Function **16**:331-337.

Li, N., N. He, G. Yu, Q. Wang, and J. Sun. 2016. Leaf non-structural carbohydrates regulated by plant functional groups and climate: Evidences from a tropical to cold-temperate forest transect. Ecological Indicators **62**:22-31.

Lovelock, C. E., J. Posada, and K. Winter. 1999. Effects of elevated CO2 and defoliation on compensatory growth and photosynthesis of seedlings in a tropical tree, Copaifera aromatica. Biotropica **31**:279–287.

McNaughton, S. J. 1983. Compensatory plant growth as a response to herbivory. Oikos **40**:329-336.

Mei, L., Y. Xiong, J. Gu, Z. Wang, and D. Guo. 2015. Whole-tree dynamics of non-structural carbohydrate and nitrogen pools across different seasons and in response to girdling in two temperate trees. Oecologia **177**:333-344.

Minchin, P. E. H., and A. Lacointe. 2005. New understanding on phloem physiology and possible consequences for modelling long-distance carbon transport. New Phytologist **166**:771–779.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science **11**:15-19.

Moore, G. M. 2013. Ring-barking and girdling: How much vascular connection do you need between roots and crown? . Pages 87-96 *in* 14th National Tree Symposium. University of Adelaide.

Niinemets, Ü. 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. Forest Ecology and Management **260**:1623-1639.

Niinemets, U., and F. Valladares. 2006. Tolerance to shade, drought, and watterlogging f temperate Northern Hemisphere trees and shrubs. Ecological Monographs **76**:521–547.

Palacio, S., R. Hernández, M. Maestro-Martínez, and J. J. Camarero. 2012. Fast replenishment of initial carbon stores after defoliation by the pine processionary moth and its relationship to the re-growth ability of trees. Trees **26**:1627-1640.

Pinkard, E. A., and C. L. Beadle. 1998. Regulation of photosynthesis in Eucalyptus nitens (Deane and Maiden) Maiden following green pruning. Trees **12**:366–376.

Popp, M., W. Lied, A. J. Meyer, A. Richter, P. Schiller, and H. Schwitte. 1996. Sample preservation for determination of organic compounds: microwave versus freeze-drying. Journal of Experimental Botany **47**:1469-1473.

Purcell, L. 2014. Mechanical Damage to Trees: Mowing and Maintenance Equipment (FNR-492-W). Forestry and Natural Resources. Purdue University.

Quentin, A. G., C. L. Beadlea, A. P. O'Gradya, and E. A. Pinkard. 2011. Effects of partial defoliation on closed canopy Eucalyptus globulus Labilladière: Growth, biomass allocation and carbohydrates. Forest Ecology and Management **261**:695-702.

Quentin, A. G., E. A. Pinkard, C. L. Beadlea, T. J. Wardlaw, A. P. O'Grady, S. Paterson, and C. L. Mohammed. 2010. Do artificial and natural defoliation have similar effects on physiology of Eucalyptus globulus Labill. seedlings? Annals of Forest Science **67**:203.

Regier, N., S. Streb, S. C. Zeeman, and B. Frey. 2010. Seasonal changes in starch and sugar content of poplar (Populus deltoides × nigra cv. Dorskamp) and the

impact of stem girdling on carbohydrate allocation to roots. Tree Physiology **30**:979-987.

Reich, P. B., M. B. Walters, S. C. Krause, D. W. Vanderklein, K. F. Raffa, and T. Tabonel. 1993. Growth, nutrition and gas exchange of Pinus resinosa following artificial defoliation. Trees 7:67-77.

Sala, A., D. R. Woodruff, and F. Meinzer. 2012. Carbon dynamics in trees: feast or famine? Tree Physiology **32**:764–775.

Sieghardt, M., E. Mursch-Radlgruber, E. Paoletti, E. Couenberg, A. Dimitrakopoulus, F. Rego, A. Hatzistathis, and T. B. Randrup. 2005. The abiotic urban environment: impact of urban growing conditions on urban vegetation. Pages 281-323 *in* C. Konijnendijk, K. Nilsson, T. Randrup, and J. Schipperijn, editors. Urban forests and trees. A feference book. Springer Verlag, Berlin.

Silpi, U., A. Lacointe, P. Kasempsap, S. Thanysawanyangkura, P. Chantuma, E. Gohet, N. Musigamart, A. Clément, T. Améglio, and P. Thaler. 2007. Carbohydrate reserves as a competing sink: evidence from tapping rubber trees. Tree Physiology **27**:881–889.

Tubby, K. V., and J. F. Webber. 2010. Pests and diseases threatening urban trees under a changing climate. Forestry **83**:451-459.

Vanderklein, D. W., and P. B. Reich. 1999. The effect of defoliation intensity and history on photosynthesis, growth and carbon reserves of two conifers with contrasting leaf lifespans and growth habits. New Phytologist **144**:121-132.

Vysotskaya, L. B., T. N. Arkhipova, L. N. Timergalina, A. V. Dedov, S. Y. Veselov, and G. R. Kudoyarova. 2004. Effect of partial root excision on transpiration, root hydraulic conductance and leaf growth in wheat seedlings. Plant Physiology and Biochemistry **42**:251–255.

Wajja-Musukwe, T. N., J. Wilson, J. I. Sprent, C. K. Ong, J. D. Deans, and J. Okorio. 2008. Tree growth and management in Ugandan agroforestry systems: effects of root pruning on tree growth and crop yield. Tree Physiology **28**:233–242.

Wiley, E., and B. R. Helliker. 2012. A re-evaluation of carbon storage in trees lends greater support for carbon limitation to growth. New Phytologist **195**:285-289.

Wiley, E., S. Huepenbecker, B. B. Casper, and B. R. Helliker. 2013. The effects of defoliation on carbon allocation: can carbon limitation reduce growth in favour of storage? Tree Physiology **00**:1-13.

GENERAL CONCLUSIONS

I proposed a new technique for measuring NSC concentrations with near-infrared spectrometry (NIRS) (***Chapter 1***). Then, I evaluated the relationship between NSC concentrations and the leaf and wood economic spectra (***Chapter 2***). And finally, I determined the response of NSC concentrations to single and interactive stress factors that are common in urban environments (***Chapters 3 and 4***).

In ***Chapter 1,*** I presented a successful application of a NIRS quantification method based on samples from many woody species, different tissue types, and a broad range of environmental conditions. The screening was done on 73 tree species and NIRS proved to be a technology with the potential to infer the concentration of NSC in a large number of samples in a rapid and inexpensive way, based on empirical calibrations with chemical analysis. Additionally, the partial least squares regression that I used to assess the relationships between NSC concentration and NIRS spectra yielded consistent, parsimonious, and robust calibrations for sugar, starch, and total NSC concentrations. This new technique for measuring NSC concentrations is a promising avenue for physiological studies that link environmental stressors and plant responses, especially after recent findings that highlighted the variability and uncertainty in accuracy in measurements of NSC among different laboratories around the world (Quentin *et al.* 2015).

Using NSC concentrations estimated for forest trees in chapter 1, I presented in ***Chapter 2*** the relationships between NSC concentrations and functional traits of temperate and tropical tree species. Contrary to our main hypothesis, the relationship between leaf functional traits and carbohydrate concentrations in stems, branches and roots was orthogonal, especially in tropical species. I found two clearly delineated orthogonal axes of variation. The first axis was formed by traits that defined the leaf economic spectrum (Wright *et al.* 2004; Chave *et al.* 2009), and a second axis was

defined by NSC concentrations. Additionally, I found weak or non-significant relationships between NSC concentrations in woody tissues and economic traits. These results suggest that an investment in traits that are associated with resource conservation, or 'slow' ecological strategies (high investment in defenses), are not related to investment in NSC storage, which provide new insights about the allocation of carbon to storage or defenses in trees with different life strategies.

In ***Chapter 3,*** I presented an assessment of the dynamics of NSC concentrations in urban trees of *Acer saccharinum* and *Acer platanoides* that immediately followed maintenance pruning. I found that pruning levels of 20-30% did not have any significant depletion effect on NSC concentrations in any tissue of either species. On the contrary, branches of pruned trees of *A. platanoides* increased NSC concentrations at the end of the growing season. These results provide valuable information for planning and management operations on urban tree populations because knowing the levels of pruning that do not decrease NSC concentrations is relevant to maintain plant tolerance to environmental constraints, and mortality (Palacio *et al.* 2008; Landhäusser & Lieffers 2012; Saffell *et al.* 2014).

In ***Chapter 4,*** I analyzed the response of NSC storage to stress in further detail, and I focused on the interactive effects of experimental defoliation, root pruning, and stem girdling on NSC concentrations and growth of *Fraxinus pennsylvanica*, *Celtis occidentalis*, and *Tilia cordata.* Although I expected a reduction in NSC concentration due to carbon limitation in the most severe three factor stress treatments, I found instead that after stress treatments, trees prioritized the maintenance of high levels of NSC concentration over growth, especially under heavily stressed treatments. The fact that trees maintain this high NSC concentrations suggest that storage competes for carbon at the expense of growth (active process).

Overall, we found that there was no clear relationship between the ecological strategies of a tree species and its investment in NSC storage (***Chapter 2***). Regardless of species functional strategies, trees increased priority of NSC storage over growth after low or high carbon-limiting conditions (active allocation to reserves) (***Chapters 3 and 4***). These results suggest that allocation of NSC to reserves has evolved as a central preventive measure to ensure long-term survival over shorter term growth (Gibon *et al.* 2009; Wiley 2013), since long-term survival depends more on the carbon available to maintain metabolism and hydraulic integrity (e.g. turgor maintenance, osmoprotection, and embolism repair; Wiley 2013) than on continuous growth.

Our results suggest a re-evaluation of the role of the carbohydrate reserves in the growth, survival and response to sudden stresses. New research efforts should focus on determining the specific function of the increased carbon allocated to reserves under severe stress events, and in establishing the physiological mechanisms that regulate such active carbon allocation to storage over growth. The use of stable carbon isotope labeling would provide information about the use of carbon between growth and storage in trees under stress conditions. Thus, the quantification of the allocation of reserves to different plant organs, as well as the ratio of growth to reserve storage through time could provide valuable information about function and control of reserves.

GENERAL REFERENCES

Atkinson, R. R. L., M. M. Burrell, K. E. Rose, C. P. Osborne, and M. Rees. 2014. The dynamics of recovery and growth: how defoliation affects stored resources. Proceedings of the Royal Society B **281**:20133355.

Canham, C. D., R. K. Kobe, E. F. Latty, and R. L. Chazdon. 1999. Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. Oecologia **121**:1-11.

Chapin, F. S., E. D. Schulze, and H. A. Mooney. 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics **21**:423-447.

Chave, J., D. Coomes, S. Jansen, S. L. Lewis, N. G. Swenson, and A. E. Zanne. 2009. Towards a worldwide wood economics spectrum. Ecology Letters **12**:351–366.

Diaz, S., J. G. Hodgson, K. Thompson, M. Cabido, J. H. C. Cornelissen, A. Jalili, G. Montserrat-Martí, J. P. Grime, F. Zarrinkamar, Y. Asri, S. R. Band, S. Basconcelo, P. Castro-Díez, G. Funes, B. Hamzehee, M. Khoshnevi, N. Pérez-Harguindeguy, M. C. Pérez-Rontomé, F. A. Shirvany, F. Vendramini, S. Yazdani, R. Abbas-Azimi, A. Bogaard, S. Boustani, M. Charles, M. Dehghan, L. de_Torres-Espuny, V. Falczuk, J. Guerrero-Campo, A. Hynd, G. Jones, E. Kowsary, F. Kazemi-Saeed, M. Maestro-Martínez, A. Romo-Díez, S. Shaw, B. Siavash, P. Villar-Salvador, and M. R. Zak. 2004. The plant traits that drive ecosystems: evidence from three continents. Journal of Vegetation Science **15**:295–304.

Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet'ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. Nature **529**:167-171.

Dietze, M. C., A. Sala, M. S. Carbone, C. I. Czimczik, J. A. Mantooth, A. D. Richardson, and R. Vargas. 2013. Nonstructural Carbon in Woody Plants. Annual Review of Plant Biology **65**:2.1–2.21.

Dong, T., B. Duan, S. Zhang, H. Korpelainen, Ü. Niinemets, and C. Li. 2016. Growth, biomass allocation and photosynthetic responses are related to intensity of root severance and soil moisture conditions in the plantation tree Cunninghamia lanceolata. Tree Physiology.

Fajardo, A., and F. I. Piper. 2014. An experimental approach to explain the Southern Andes elevational treeline. American Journal of Botany **101**:788–795.

Ferree, D. C., D. M. Scurlock, and J. C. Schmid. 1999. Root pruning reduces photosynthesis, transpiration, growth, and fruiting of container-grown French-American hybrid grapevines. HortScience **34**:1064–1067.

Gibon, Y., E. Pyl, R. Sulpice, J. Lunn, M. Hohne, M. Gunther, and M. Stitt. 2009. Adjustment of growth, starch turnover, protein content and central metabolism to a decrease of the carbon supply when Arabidopsis is grown in very short photoperiods. Plant Cell and Environment **32**:859–874.

Gleason, S. M., and A. Ares. 2004. Photosynthesis, carbohydrate storage and survival of a native and an introduced tree species in relation to light and defoliation. Tree Physiology **24**:1087-1097.

Grime, J. P., K. Thompson, R. Hunt, J. G. Hodgson, J. H. C. Cornelissen, I. H. Rorison, G. A. F. Hendry, T. W. Ashenden, A. P. Askew, S. R. Band, R. E. Booth, C. C. Bossard, B. D. Campbell, J. E. L. Cooper, A. W. Davison, P. L. Gupta, W. Hall, D. W. Hand, M. A. Hannah, S. H. Hillier, D. J. Hodkinson, A. Jalili, Z. Liu, J. M. L. Mackey, N. Matthews, M. A. Mowforth, A. M. Neal, R. J. Reader, K. Reiling, W. Ross-Fraser, R. E. Spencer, F. Sutton, D. E. Tasker, P. C. Thorpe, and J. Whitehouse. 1997. Integrated screening validates primary axes of specialisation in plants. Oikos **79**:259–281.

Handa, T., C. Körner, and S. Hättenschwiler. 2005. A test of the treeline carbon limitation hypothesis by in situ CO2 enrichment and defoliation. Ecology **86**:1288-1300.

Hoch, G., and C. Körner. 2012. Global patterns of mobile carbon stores in trees at the high-elevation tree line. Global Ecology and Biogeography **21**:861-871.

Hoch, G., A. Richter, and C. Körner. 2003. Non-structural carbon compounds in temperate forest trees. Plant, Cell and Environment **26**:1067-1081.

Högberg, P., A. Nordgren, N. Buchmann, A. F. S. Taylor, A. Ekblad, M. N. Högberg, G. Nyberg, M. Ottosson-Löfvenius, and D. J. Read. 2001. Large-scale forest girdling shows that current photosynthesis drives soil respiration. Nature **411**:789–792.

Jacquet, J. S., A. Bosc, A. O'Grady, and H. Jactel. 2014. Combined effects of defoliation and water stress on pine growth and non-structural carbohydrates. Tree Physiology **00**:1–10.

Kobe, R. K. 1997. Carbohydrate allocation to storage as a basis of interspecific variation in sapling survivorship and growth. Oikos **80**:226-233.

Konijnendijk, C. C., and T. B. Randrup. 2004. Urban forestry. Pages 471-478 *in* J. Burley, J. Evans, and J. Youngquist, editors. Encyclopedia of Forest Sciences. Academic Press, Amsterdam.

Körner, C. 2003. Carbon limitation in trees. Journal of Ecology **91**:4-17.

Lacointe, A. 2000. Carbon allocation among tree organs: a review of basic processes and representation in functional-structural tree models. Annals of Forest Science **57**:521–533.

Landhäusser, S. M., and V. J. Lieffers. 2003. Seasonal changes in carbohydrate reserves in mature northern Populus tremuloides clones. Trees **17**:471–476.

Landhäusser, S. M., and V. J. Lieffers. 2012. Defoliation increases risk of carbon starvation in root systems of mature aspen. Trees **26**:653–661.

Li, M. H., G. Hoch, and C. Körner. 2002. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. Trees-Structure and Function **16**:331-337.

McDowell, N., W. T. Pockman, C. D. Allen, D. D. Breshears, N. Cobb, T. Kolb, J. Plaut, J. Sperry, A. West, D. G. Williams, and E. A. Yepez. 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? New Phytologist **178**:719-739.

Mei, L., Y. Xiong, J. Gu, Z. Wang, and D. Guo. 2015. Whole-tree dynamics of non-structural carbohydrate and nitrogen pools across different seasons and in response to girdling in two temperate trees. Oecologia **177**:333-344.

Minchin, P. E. H., and A. Lacointe. 2005. New understanding on phloem physiology and possible consequences for modelling long-distance carbon transport. New Phytologist **166**:771–779.

Mitchell, P. J., A. P. O'Grady, D. T. Tissue, D. A. White, M. L. Ottenschlaeger, and E. A. Pinkard. 2013. Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. New Phytologist **197**:862-872.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends in Plant Science **11**:15-19.

Moore, G. M. 2013. Ring-barking and girdling: How much vascular connection do you need between roots and crown? . Pages 87-96 *in* 14th National Tree Symposium. University of Adelaide.

Myers, J. A., and K. Kitajima. 2007. Carbohydrate storage enhances seedling shade and stress tolerance in a neotropical forest. Journal of Ecology **95**:383-395.

Niinemets, Ü. 2010. Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. Forest Ecology and Management **260**:1623-1639.

Niinemets, U., and F. Valladares. 2006. Tolerance to shade, drought, and watterlogging f temperate Northern Hemisphere trees and shrubs. Ecological Monographs **76**:521–547.

O'Brien, M. J., S. Leuzinger, C. D. Philipson, J. Tay, and A. Hector. 2014. Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. Nature Climate Change **4**:710-714.

Palacio, S., A. J. Hester, M. Maestro, and P. Millard. 2008. Browsed *Betula pubescens* trees are not carbon-limited. Functional Ecology **22**:808-815.

Poorter, L., and K. Kitajima. 2007. Carbohydrate storage and light requirements of tropical moist and dry forest tree species. Ecology **88**:1000-1011.

Purcell, L. 2014. Mechanical Damage to Trees: Mowing and Maintenance Equipment (FNR-492-W). Forestry and Natural Resources. Purdue University.

Quentin, A. G., C. L. Beadlea, A. P. O'Gradya, and E. A. Pinkard. 2011. Effects of partial defoliation on closed canopy Eucalyptus globulus Labilladière: Growth, biomass allocation and carbohydrates. Forest Ecology and Management **261**:695-702.

Quentin, A. G., A. P. O'Grady, C. L. Beadle, C. Mohammed, and E. A. Pinkard. 2012. Interactive effects of water supply and defoliation on photosynthesis, plant water status and growth of Eucalyptus globulus Labill. Tree Physiology **00**:958–967.

Quentin, A. G., E. A. Pinkard, C. L. Beadlea, T. J. Wardlaw, A. P. O'Grady, S. Paterson, and C. L. Mohammed. 2010. Do artificial and natural defoliation have similar effects on physiology of Eucalyptus globulus Labill. seedlings? Annals of Forest Science **67**:203.

Quentin, A. G., E. A. Pinkard, M. G. Ryan, D. T. Tissue, L. S. Baggett, H. D. Adams, P. Maillard, J. Marchand, S. M. Landhäusser, A. Lacointe, Y. Gibon, W. R. L. Anderegg, S. Asao, O. K. Atkin, M. Bonhomme, C. Claye, P. S. Chow, A. Clément-Vidal, N. W. Davies, L. T. Dickman, R. Dumbur, D. S. Ellsworth, K. Falk, L. Galiano, J. M. Grünzweig, H. Hartmann, G. Hoch, S. Hood, J. E. Jones, T. Koike, I. Kuhlmann, F. Lloret, M. Maestro, S. D. Mansfield, J. Martínez-Vilalta, M. Maucourt, N. G. McDowell, A. Moing, B. Muller, S. G. Nebauer, Ü. Niinemets, S. Palacio, F. Piper, E. Raveh, A. Richter, G. Rolland, T. Rosas, B. Saint Joanis, A. Sala, R. A. Smith, F. Sterck, J. R. Stinziano, M. Tobias, F. Unda, M. Watanabe, D. A. Way, L. K. Weerasinghe, B. Wild, E. Wiley, and D. R. Woodruff. 2015. Non-structural carbohydrates in woody plants compared among laboratories. Tree Physiology.

Ramirez, J. A., J. M. Posada, I. T. Handa, G. Hoch, M. Vohland, C. Messier, and B. Reu. 2015. Near-infrared spectroscopy (NIRS) predicts non-structural carbohydrate concentrations in different tissue types of a broad range of tree species. Methods in Ecology and Evolution **6**:1018–1025.

Reich, P. B. 2014. The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. Journal of Ecology **102**:275–301.

Reich, P. B., M. B. Walters, S. C. Krause, D. W. Vanderklein, K. F. Raffa, and T. Tabonel. 1993. Growth, nutrition and gas exchange of Pinus resinosa following artificial defoliation. Trees 7:67-77.

Saffell, B. J., F. C. Meinzer, D. R. Woodruff, D. C. Shaw, S. L. Voelker, B. Lachenbruch, and K. Falk. 2014. Seasonal carbohydrate dynamics and growth in Douglas-fir trees experiencing chronic, fungal-mediated reduction in functional leaf area. Tree Physiology.

Sieghardt, M., E. Mursch-Radlgruber, E. Paoletti, E. Couenberg, A. Dimitrakopoulus, F. Rego, A. Hatzistathis, and T. B. Randrup. 2005. The abiotic urban environment: impact of urban growing conditions on urban vegetation. Pages 281-323 *in* C. Konijnendijk, K. Nilsson, T. Randrup, and J. Schipperijn, editors. Urban forests and trees. A feference book. Springer Verlag, Berlin.

Tello, M. L., M. Tomalak, R. Siwecki, J. Gáper, E. Motta, and E. Mateo-Sagasta. 2005. Biotic Urban Growing Conditions –Threats, Pests and Diseases.*in* C. C. Konijnendijk, K. Nilsson, T. B. Randrup, and J. Schipperijn, editors. Urban Forests and Trees. Springer-Verlag, Berlin.

Tubby, K. V., and J. F. Webber. 2010. Pests and diseases threatening urban trees under a changing climate. Forestry **83**:451-459.

Vanderklein, D. W., and P. B. Reich. 1999. The effect of defoliation intensity and history on photosynthesis, growth and carbon reserves of two conifers with contrasting leaf lifespans and growth habits. New Phytologist **144**:121-132.

Vysotskaya, L. B., T. N. Arkhipova, L. N. Timergalina, A. V. Dedov, S. Y. Veselov, and G. R. Kudoyarova. 2004. Effect of partial root excision on transpiration, root hydraulic conductance and leaf growth in wheat seedlings. Plant Physiology and Biochemistry **42**:251–255.

Wajja-Musukwe, T. N., J. Wilson, J. I. Sprent, C. K. Ong, J. D. Deans, and J. Okorio. 2008. Tree growth and management in Ugandan agroforestry systems: effects of root pruning on tree growth and crop yield. Tree Physiology **28**:233–242.

Westoby, M., D. S. Falster, A. T. Moles, P. A. Vesk, and I. J. Wright. 2002. Plant ecological strategies: some leading dimensions of variation between species. Annual Review of Ecology and Systematics **33**:125-159.

Wiley, E. 2013. Towards A Better Understanding Of Nonstructural Carbohydrate Storage And Carbon Limitation In Trees. University of Pennsylvania.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M.-L. Navas, U. l. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The world-wide leaf economics spectrum. Nature **428**:821–827.

Würth, M. K. R., S. Pelaez-Riedl, S. J. Wright, and C. Körner. 2005. Non-structural carbohydrate pools in a tropical forest. Oecologia **143**:11–24.

Zhang, H., C. Wang, and X. Wang. 2014. Spatial variations in non-structural carbohydrates in stems of twelve temperate tree species. Trees **28**:77–89.