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ROOT CARBOHYDRATE STORAGE IN TEMPERATE AND TROPICAL FOREST TREE SEEDLINGS: IMPLICATIONS FOR SPECIES COEXISTENCE

By

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ABSTRACT

ROOT CARBOHYDRATE STORAGE IN TEMPERATE AND TROPICAL FOREST TREE SEEDLINGS: IMPLICATIONS FOR SPECIES COEXISTENCE

By

Meera Iyer

With the goal of better understanding the functional significance of intra- and inter-specific variation in plant resource allocation, its implications for species growth and survival and hence for community composition, I carried out experiments with tree seedlings from both temperate and tropical forest communities. In Michigan, seedlings of eight common northern temperate species were grown in a greenhouse under two levels of light and nitrogen availability, spanning the range of variation found in northern Michigan forests. In the tropics, I transplanted ~ 3,000 seedlings of five common dry tropical forest tree species into natural gradients of light and soil phosphorus (P) availability in Palo Verde National Park, Costa Rica. Light availability in the plots ranged from ~4% to ~40% full sun, and phosphorus availability, from <1 to ~150 mg P/kg soil. In both the tropical and temperate experiments, seedlings were harvested at intervals throughout the experiment and their root morphology and allocation to root total nonstructural carbohydrate (TNC) storage measured. Across species and biomes, stored root reserves accounted for 8 - 60% of root dry mass. TNC increased with increased light availability and with decreased nutrient availability. In both temperate and tropical species, root TNC clearly drives changes in root mass ratio (RMR) while resource-driven changes in structural root mass are absent in most species and weak in others. Hence, my results suggest that the increased RMR associated with lower nutrient availability – usually interpreted as an increased allocation towards nutrient capture – is unlikely to lead to increased nutrient uptake since it is due largely to increased storage. In Michigan, root carbohydrate storage was negatively correlated with seedling growth rates and positively correlated with increased survival of field seedlings. In the tropics, there was a positive correlation between root TNC and growth in the high soil P site and a negative correlation in the low P site. Contrary to expectations, survival and root TNC were not positively linked in the tropics. There was an interspecific trade-off between species growth under high resource availability and survival under low resource availability in the high soil P site but no trade-off in the low soil P site. The competitive hierarchy of species changed with both light and soil resource levels, and suggested resource gradient partitioning among regenerating seedlings of these dry forest species.

For my parents and for Sridhar

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Chapter 1

Resource allocation and its implications for species growth and survival

Introduction

Elucidating mechanisms that maintain species diversity in ecological communities has long been a goal in ecology. Several hypotheses have been put forward to explain coexistence in forests (Chesson 2000). Of these, the idea of niche partitioning has been the focus of much research. This hypothesis considers that differences among species in resource use, coupled with spatial and temporal variations in availability of these resources, can lead to species coexistence (Denslow 1987). An alternative to niche theory, the unified neutral theory of biodiversity, calls into question the central assumptions of niche partitioning and asserts that species are competitively equivalent (Hubbell 2001). Analogous to the neutral theory of molecular evolution (Kimura 1983), the neutral theory of biodiversity states that species diversity and relative abundances are determined by random outcomes of probabilistic demographic processes (Caswell 1976, Hubbell 1979, Hubbell 2001). The neutral model successfully predicts species composition patterns in communities (Hubbell 2001). However, the same patterns are also predicted by non-neutral models, and a strict assumption of species equivalence finds little empirical support (Chave 2002, Condit et al. 2002, Fargione et al. 2003, Silvertown 2004) and is contrary to the numerous studies that have found associations between species traits, species composition and environmental conditions (e.g., Grime 1979, Tilman 1988, Pacala et al. 1996, Kobe 1999, Rees et al. 2001). It is likely that both niche partitioning and stochastic processes contribute to species coexistence (e.g., Tilman 2004), though the relative importance of each in particular communities remains unknown.

Light availability is limited in most ecosystems where research on species composition has been carried out, including humid tropical forests and most temperate forests. Hence, the majority of niche partitioning studies have focused on partitioning of light availability, disregarding the role of soil resources, which are also critical to plant growth and survival (Marschner 1985) and, like light availability, vary spatially (Stoyan et al. 2000, Sollins 1998) and temporally (Lodge et al. 1994, Campo et al. 1998). Such heterogeneity in soils, together with different species responses to soil resource conditions (Burns and Honkala 1990, Sollins 1998) leads to species segregation along topographic or edaphic gradients in both tropical (Davies 2001) and temperate forests (Host & Pregitzer 1992) and soil-based habitat specialization in both biomes (Stoyan et al. 2000, Hall et al. 2004, Palmiotto et al. 2004). Species differences in performance under diverse soil resource regimes (Kobe 1996, Schreeg et al. 2005) suggest that belowground resources may also be subject to niche partitioning. Furthermore, niche differences could operate on two axes simultaneously because resources allocated towards light-harvesting structures are unavailable for soil-resource capture (King 1993).

Differences in species performance under different resource environments is a fundamental tenet of niche theory, which requires that no one species is the best competitor under all resource regimes. In this view, species coexistence is facilitated by trade-offs in species performance under differing environmental conditions. Several studies have demonstrated that across light environments, there is an interspecific trade-off between survivorship under low light availability and growth under high light availability in tropical (Hubbell and Foster 1992, Kobe 1999) and temperate forests (Kobe et al. 1995, Lin et al. 2002). This trade-off facilitates species coexistence in temperate forests (Pacala et al. 1996). Recent research in northern Michigan shows a similar trade-off operates via soil resources so that species that grow rapidly under high fertility survive poorly under conditions of low soil resource availability; this trade-off likely contributes to differences in species composition seen across broad landscape-level gradients of soil resource availability (Schreeg et al. 2005).

What are the mechanisms that allow rapid growth under abundant resource availability, but preclude survival in resource-poor environments? The allocation of photosynthate to different functions within the plant could be one mechanism that defines differences in species' growth and survival across resource environments. Research on allocation has concentrated on the effects of light and soil-resource availability on allocation to light-harvesting structures, i.e., to leaves. For example, at high resource availability, species adapted to those conditions have greater leaf surface area than species adapted to poor sites (Poorter and De Jong 1999, Craine et al. 2001), with the increased leaf area brought about by a combination of morphological changes at the leaf (thinner leaves) and whole-plant level (increased fraction of plant mass in leaves).

Relatively less research has been focused on the effects of resource availability on allocation to soil nutrient and water harvesting structures, i.e., to roots. Analogous to the approach taken with leaves, allocation to roots has been investigated at the whole plant level by studying root mass fraction (the fraction of whole plant mass that is allocated to roots), and at the morphological level by studying the surface area or length of roots. Thus, most species in low-nutrient environments allocate a greater portion of mass to roots than to shoots, leading to a higher root mass fraction than stem mass fraction

(Reynolds and D'Antonio 1996, Aerts and Chapin 2000). Again, species from more fertile habitats, which typically have faster growth rates, usually have higher specific root surface area and specific root length than those from low-soil resource environments when grown under the same conditions (Reich et al. 1998, Craine et al. 2001, Comas et al. 2002). Thus, fast-growing species often have a greater capacity to capture resources through greater allocation to resource-harvesting structures, and increase allocation to these structures as resource availability increases, though a link between growth rate, habitat association and root morphology is not always apparent (Poorter and Remkes 1990).

However, a direct analogy between allocation to leaves and roots is facile because roots, unlike leaves, are often the major site of carbohydrate storage (Loescher et al. 1990). Up to 40% of root mass can consist of nonstructural carbohydrates (Singh and Srivastava 1986, Nguyen et al. 1990, Canham et al. 1999, Newell et al. 2002). Moreover, the failure to distinguish between storage and non-storage tissue could lead to misinterpretations of patterns of allocation since allocation to storage has very different outcomes than allocation to structural growth.

Storage in the form of total nonstructural carbohydrates (TNC) can occur when there is asynchrony in carbon supply and demand (Chapin et al. 1990). It can also compete directly with allocation towards resource-harvesting structures, and hence with growth (Chapin et al. 1990). Regardless of the cause of reserve formation, stored carbohydrates can be essential for survival (Kozlowski 1992) especially when there is seasonal variation in resource supply (Chapin et al. 1990). For instance, in temperate climates, TNC allows maintenance respiration over the winter, confers frost resistance and is essential for regrowth following periods of deciduousness (Chapin et al. 1990, Kozlowski 1992). TNC can also play an important role in recovery from herbivory in both tropical (Marquis et al. 1997) and temperate systems (Webb 1981) and may be an important carbon source for fine root growth in later years (Langley et al. 2002). TNC is particularly important in environments experiencing periodic disturbances (Sakai et al. 1997, Iwasa and Kubo 1987) and may allow some species to resprout following disturbance (El Omari et al. 2003, Hoffmann et al. 2004).

Allocation to storage can vary with resource environment (Mooney et al. 1995, Gansert and Sprick 1998) and among species (Kobe 1997, Canham et al. 1999, Newell et 2002, Wurth et al. 2005). Previous research has suggested a link between TNC and survival, particularly under low light (Kobe 1997) and is also important in drought tolerance (Kozlowski and Pallardy 2002). Allocation to TNC thus appears to promote survival, often at the expense of growth, and is likely to be particularly important in species that emphasize survival over growth, especially under low resource availability. Allocation to carbohydrate storage could thus be an important physiological mechanism underlying the documented trade-off between low-resource survival and high-light growth: species that have an allocation pattern that promotes growth could be expected to have lower allocation to storage, and hence lower survival under low resource environments.

Research objectives

The objectives of my research are to investigate inter- and intra- specific variation in root biomass allocation patterns, characterizing allocation to stored reserves and to non-storage tissue across gradients of light and nutrient availability. A more physiologically-based understanding of species differences in competitive ability, growth and survival under different resource environments would derive insight into mechanisms determining species composition in forest communities, an overarching goal of my project. To test the generality of these ideas, my research critically examines allocation patterns and their relation to species performance across resource regimes in two different biomes – northern temperate forests of Michigan and dry tropical forests of Costa Rica, the latter considered the most endangered of all tropical habitats.

Dissertation outline

This dissertation presents an integrated set of greenhouse experiments, field experiments, and modeling to evaluate the role of carbohydrate storage as a basis for ecological differentiation and hence community composition in Michigan and Costa Rica. Chapters 2 and 3 are based on an experiment with seedlings of 8 common northern temperate species that were grown at two light levels and two nitrogen levels in a greenhouse environment for four months. Chapter 2 focuses on intra-specific variation in allocation to root carbohydrate storage or non-storage tissue in response to resource availability, and demonstrates that increases in root mass due to low soil resources or high light are likely driven by TNC accumulation rather than increased allocation to resource-harvesting fine roots, which is how variation in root mass allocation has been previously interpreted. Chapter 3 is focused on interspecific variation in TNC-structural tissue allocation in roots and the implications of differing allocation patterns on species growth and survival. In particular, I found that allocation to TNC could explain the trade-off between growth under high resource availability and survival under low resource availability across northern temperate forest tree species. Chapters 4 and 5 deal with field experiments in a dry tropical forest in Costa Rica, where seedlings of five tree species were transplanted into plots stratified across light and soil resource gradients. Chapter 4 focuses on seedling survival and growth and describes partitioning of soil resource and light availability by the seedlings of these tree species. I found a species trade-off between high light growth and low light survival, but only when soil resource availability is high. Chapter 5 investigates root TNC stores in the five species and discusses its relationships with species growth and survival. Species allocation to storage traded-off with growth only when soil resource availability was low but was positively linked to growth when soil resource availability was high. In all chapters, I used maximum likelihood techniques to generate predictive models of the effects of resource availability on species-specific allocation to root storage or non-storage tissue, and on survival and growth. My research hence attempts an understanding of community organization through critically evaluating species differences in performance and the physiological mechanisms that cause those differences.

Chapter 2

Storage of nonstructural carbohydrates drives seedling root mass responses to light and nitrogen

Introduction

The allocation of limited resources to different functions within a plant underlies species-specific performance across resource environments. Much is known about mass allocation among organs (e.g., Poorter and De Jong 1999, Walters and Reich 1999). For instance, most species respond to decreasing light availability by increasing leaf area through some combination of morphological changes at both the leaf (thinner leaves) and whole- plant levels (increased fraction of plant mass in leaves). In contrast to leaves, species-specific responses of root structure and function to changing resource environments are only beginning to be understood (Ryser 1998, Comas et al. 2002). However, the allocation of carbon within organs into various compounds affects the growth and functioning of the plant (e.g., Bazzaz et al., 1987). Once again, allocation within leaves has received more attention than roots, especially among herbaceous species. For instance, the principal carbon compounds in leaves of herbaceous species and fast-growing woody species are proteins, accounting for 27%, followed by structural compounds such as cellulose and hemicelluose, and total nonstructural carbohydrates, accounting for 14% each of dry mass (Poorter et al. 1997). In contrast, although storage reserves and concentrations of structural compounds are thought to be higher, and of proteins, organic acids and lipids lower, in roots than in leaves, especially in woody species, these generalizations are based on very few studies (Poorter and Villar 1997).

In roots, non-structural carbohydrate storage can constitute between 10 and 40% of total root dry mass (Nguyen et al. 1990, Kobe 1997, Canham et al. 1999). Although any perennial plant organ may serve as a storage reservoir, roots often serve as the primary storage site and have the highest concentrations of TNC (Loescher et al. 1990) , perhaps because they are less likely to be damaged or destroyed by disturbances such as treefalls or fire. Allocation to storage is especially important when resource availability varies temporally (Chapin et al. 1990). In temperate regions, for instance, where carbon gain for deciduous trees is limited to the growing season, carbohydrate storage is essential for the maintenance of living tissue over winter, for making leaves the following spring (Loescher et al. 1990, Kozlowski 1992) and for fine root growth in later years (Langley et al. 2002). In deciduous trees, carbon reserves generally reach a maximum at the end of the growing season, slowly deplete during the dormant season, rapidly deplete during new leaf and fine root flushes, and rebuild throughout the growing season (Loescher et al. 1990, Kozlowski 1992, Newell et al. 2002, Gaucher et al. 2005). Allocation to storage also varies with resource availability. Stored carbon generally increases with higher light availability (Mooney et al. 1995, Naidu and DeLucia 1997, Gansert and Sprick 1998) and with lower nutrient availability (McDonald et al. 1986, Fichtner et al. 1993, Mooney et al. 1995, Paul and Driscoll 1997). By buffering the environment, stored carbohydrates can also enhance survival, enabling recovery after defoliation due to herbivory or disease (Kobe 1997, Marquis et al. 1997, Canham et al. 1999). Furthermore, there is considerable interspecific variation in allocation to storage (Kobe 1997, Canham et al. 1999, Newell et al. 2002, Iyer et al.) which may be related to variation in adaptive strategies among species. For example storage may allow shade tolerant species in understory environments opportunistic growth spurts following canopy opening (DeLucia et al. 1998, Gaucher et al. 2005), and disturbance adapted species to resprout following top-kill (Iwasa and Kubo 1987, El Omari et al. 2003).

Although both storage and non-storage compounds serve multiple functions within the plant, allocation to non-storage tissue implies an increased plant investment in one or all of the following: increased access to resources, via increased allocation to root structural compounds; increased soil resource uptake via increased allocation to compounds involved in metabolism, such as lipids and proteins, or to soluble carbohydrates which are also involved in supporting mycorrhizae; and increased defense via increases in lignins and phenolics (Poorter and Villar 1997). Fine roots are a plant's interface with soil and enable access to and uptake of nutrients and water. Though most studies have not differentiated between storage and other pools, fine roots involved in nutrient capture typically contain only 4-6% TNC (Pregitzer et al. 2000). In general, fine root production (and mortality) increases with nitrogen availability (Pregitzer et al. 1993, Van Vuuren et al. 1996, Espeleta and Donovan 2002). Because resource availability influences fine root dynamics, which are largely composed of non-storage tissue, allocation to non-storage mass in roots should be strongly influenced by resources.

Given that root storage and non-storage tissue vary independently with resources and across species, failing to take stored carbohydrates into account could lead to erroneous conclusions about intra- and inter- specific variation in root characteristics. For example, root morphological metrics typically are normalized by total root mass (and hence include nonstructural carbon pools). However, although they can be mobilized, stored carbohydrates are resources sequestered mostly for future needs, rather than for current use so that the inclusion of stored carbon mass in metrics such as specific root area can lead to biased estimates of allocation to nutrient capture. A more functionally realistic index of allocation to resource capture should differentiate between stored reserves and structural biomass. We define root structural mass as the difference between root mass and carbohydrate stores in the root (Canham et al. 1999), recognizing that this is a simplistic differentiation given that both storage and non-storage carbon can serve multiple functions in the plant.

To test the effects of resource levels on intra-specific variation in dry mass allocation to root storage and structural tissue, we carried out a greenhouse experiment with seedlings of eight temperate tree species. In particular, we tested the hypothesis that effects of light and nitrogen on plant allocation to root mass are primarily driven by changes in allocation to nonstructural carbohydrates rather than by changes in structural root mass that enhance resource harvesting. Because structural root mass alone is a crude metric of the potential to take up soil resources, we tested that the conversion of structural root mass to area becomes more efficient (i.e, results in higher root surface areas) with decreasing nitrogen and increasing light availability. Finally, because most studies on root function have not distinguished between nonstructural and structural carbon pools, we tested the hypothesis that normalizing root morphological traits such as surface area to total root mass rather than structural root mass decreases sensitivity to detecting resource effects because under high light or low N, increased root mass due to TNC accumulation would cancel out any increases in root length or surface area.

Materials and methods

Species and seed sources

Our study species were *Acer rubrum* L. (red maple), *A. saccharum* Marsh. (sugar maple), *Quercus velutina* Lam. (black oak), *Q. rubra* L. (red oak), *Q. alba* L.(white oak), *Prunus serotina* Ehrh. (black cherry), *Fagus grandifolia* Ehrh. (American beech) and *Betula* *papyrifera* Marsh. (paper birch). Collectively, they encompass a wide range of shade tolerances and associations with soil resource levels (Table 2.1). Seeds for all the species were purchased from Sheffield Seed Company, Locke, NY, USA.

Growth media, light and nutrient levels

Seeds were stratified and then germinated in perlite. In February 2002, germinants were planted in polyethylene-coated 10.2 cm x 10.2 cm x 27 cm cardboard containers filled with a 10:9:1 (v:v:v) of a silica sand:perlite:field soil mix. The field soil was obtained from a mesic beech-maple-oak forest near the MSU Tree Research Center, East Lansing. This mixture provided a relatively inert, nutrient poor medium where nutrient additions could be controlled. It also facilitated recovery of fine roots during harvests.

The experiment was designed as a 2 x 2 factorial with two levels of light (~2% and ~22% of open-sky light) and nitrogen availability (0.5 mg N l^{-1} and 50 mg N l^{-1} in a modified Hoagland's solution added every three days). To prevent buildup of salts, all containers were flushed with deionized water weekly. Light levels were designed to mimic endpoints in the range of light conditions from understorey to tree fall gaps encountered in northern lower Michigan forests (Schreeg et al. 2005). Similarly, the high N treatment approximates available nitrogen levels in high fertility moraines in Manistee National Forest in northern lower Michigan (~7 µg N g^{1} soil; Zak et al. 1986, Kobe, unpublished data). To achieve light levels, we used an inner layer of black shade cloth combined with an outer layer of reflective knitted poly-aluminum shade cloth, the latter used to minimize heat build-up. Temperatures in each shade treatment were monitored with Hobo dataloggers (Onset Computer Corporation, Bourne, MA, USA) and were found not to differ (t test, $p > 0.95$). Mean daytime temperatures over the experiment in the two treatments were 23.63±0.04 °C and 23.61±0.04 °C in the high and low light treatments respectively.

Under high light, we planted 30 - 36 seedlings in each species x nutrient level combination. To compensate for expected higher mortality under low light, we planted 36 - 45 seedlings in each species x nutrient level combination. The experiment began in the first week of February 2002 and continued through July 2002.

Root morphology

To investigate intra- and inter-specific variation in root storage and structural pools and their ontogeny, we harvested subsets of seedlings at regular intervals to 3 months (Table 2.1). At each harvest, six individuals from each species-nutrient-light combination were harvested. Some harvests could not be carried out due to mortality, especially under low light treatments. To minimize variation among samples due to diurnal patterns in carbohydrate storage, all harvests were initiated 2.5 hours after sunrise. Seedlings were washed in deionized water, separated into leaves, stems and roots. Fresh roots were scanned and the digital images later analyzed for root length and surface area with WinRhizo (Regent Instruments, Blain, Quebec, Canada). All plant parts were freeze-dried for 2 days and then weighed. Dried roots were pulverized with a ball mill (Kinetic Laboratory Equipment Co., California) before TNC extraction and analysis.

Table 2.1 Species used in the experiment, with their shade tolerance, soil resource affinities, and their age at harvests. Shade tolerance categories are based on Burns and Honkala (1990).

Species	**Common name**	**Abbre-viation**	**Shade tolerance category**	**Soil resource affinity**	**Age at harvest (days)**			
					1	**2**	**3**	**4**
Acer rubrum	Red maple	RM	tolerant	inter-mediate	-	34	75	105
A. saccharum	Sugar maple	SM	very tolerant	high fertility	-	37	86	-
Quercus alba	White oak	WO	intolerant	low fertility	10	37	76-77	106
Q. velutina	Black oak	BO	intolerant	low fertility	-	-	84	105
Q. rubra	Red oak	RO	intermediate	low – high fertility	14	40	85	106
Prunus serotina	Black cherry	BC	intolerant	high fertility	25	53	86	106
Fagus grandifolia	American beech	AB	very tolerant	intermediate fertility	29	52	84-85	105
Betula papyrifera	Paper birch	PB	intolerant	high fertility	-	-	-	122

Table 2.2 Candidate models characterizing functional relationship between root stored reserves or structural biomass (y) and whole plant biomass (x). Models 1-3 were also used to characterize relationships between fine root surface area (y) and structural root mass or total root mass (x).

Model	Equation	Interpretation
1	$y = ax$	Proportional increases ($a > 0$) or decreases ($a < 0$) in TNC (or structural biomass) with whole plant mass (WPM)
2	$y = exp(a*x) - 1$	Exponential increases ($a > 0$) or decreases ($a < 0$) in TNC (or structural biomass) with WPM
3	$y = a*(x\hat{}b)$	More than proportional increases ($b > 1$) or less than proportional increases ($b < 1$) in TNC (or structural biomass) with WPM
4	$y = a*exp(-b/x)$	Increases in TNC (or structural biomass) with WPM follows a sigmoidal curve

Carbohydrate analyses

We used a two-step process to measure TNC. First, we extracted and analyzed soluble sugars from tissues, then analyzed extraction residues for starch. Because soluble sugars usually serve multiple physiological functions in the plant besides storage (Chapin et al. 1990), starch is often considered the most important reserve carbohydrate and is used as the sole indicator of stored reserve status (Kozlowski 1992). We chose a two-step process to enable us to measure levels of soluble sugars and starch separately. We determined TNC in roots of all seedlings harvested. For a subset of the species, we also analyzed TNC levels in stems at the final harvest, enabling us to determine whole plant level concentrations. We did not analyze carbohydrate levels in leaves because we were interested in longer-term stores rather than diurnal pools of sugars (Schnyder 1993).

Soluble carbohydrates in a 20mg sample were extracted three times at 75°C using 2ml of 80% ethanol and then centrifuged at 1900 g for 5 minutes. The supernatants were collected and diluted to a known volume with deionized water and the concentration of soluble sugars (as glucose equivalents) was measured at 490 nm using a phenol-sulfuric acid colorimetric assay (Dubois et al. 1956). The phenol-sulfuric acid assay does not detect sugar alcohols such as sorbitol, an important soluble carbohydrate in some *Prunus* sp. (Keller and Loescher 1989). To determine sugar alcohols, black cherry alcohol extracts were dried and resuspended in 1 mL of pyridine containing 30 mg ml^{-1} hydroxylamine hydrochloride and β-phenyl-D-glucoside as an internal standard. The suspension was heated at 75°C for 1 h, derivatized using a combination of hexamethyldisilazane and trifluoroacetic acid (Sweeley et al. 1963) and then analyzed using gas chromatography (Roper et al. 1988).

The pellet remaining after ethanol extraction was dried and quantitatively analyzed for starch. To gelatinize the starch, we added 2 ml of 0.1 M sodium acetate buffer (pH 5) and autoclaved the sample at 125°C for 10 minutes. After cooling, the sample was incubated with 10 units of amyloglucosidase (Roche Diagnostics Corp., Indianapolis, IN, USA) at 55°C for 16 hours. Because sample processing can introduce trace amounts of monomers derived from structural carbohydrates, the extractant was analyzed colorimetrically using glucose-specific trinder reagent (Sigma Chemical Co., St Louis, MO, USA) (Roper et al. 1988).

TNC concentration was calculated as the sum of glucose equivalents of soluble sugars and starch measured in each sample. We also calculated pool sizes (concentration x root mass) of stored carbohydrates. Concentration is a good measure of proportional allocation to storage, whereas pool sizes estimate total reserves available for future use on a whole-plant basis (Chapin et al. 1990). For some species such as black cherry and sugar maple, individuals grown under low light were too small for TNC analysis. Individual samples in these treatments were combined to obtain one or more composite samples with sufficient mass for analysis. No paper birch seedlings under low light were available for analysis due to high mortality.

Models and data analysis

We used a set of candidate models to evaluate how root TNC and non-storage mass (= total root mass – TNC pool) varies as a function of whole plant mass (Table 2.2). A linear model with no constant (Model 1) characterizes a constant fraction of whole plant mass being allocated to root storage (or non-storage mass). Model 2 represents an exponential increase in allocation to storage (or non-storage mass) with an increase in whole plant mass. Model 3 is the commonly used allometric scaling model (Niklas and Enquist 2001, Kobe et al. 2005) where $b > 1$ implies allocation to root TNC (or structure) increases disproportionately with whole plant mass while $b < 1$ implies less than proportionate increases with plant size. Model 4, a sigmoidal curve, characterizes a lag in allocation to storage (or non-storage mass) at small sizes with increased allocation as size increases, as has been shown under low light in some species (e.g., Kabeya and Sakai 2003). The a parameter represents the asymptotic TNC (or non-storage) pool over the range of whole plant mass in our experiment and b represents the rate of increase of TNC (or non-storage root mass) with whole plant mass. Model parameters were estimated using maximum likelihood methods (Hilborn and Mangel 1997).

We tested effects of light and N on TNC, independent of total plant mass effects, by using dummy variables for discrete light and N treatments. For example, for a linear relationship between root TNC pool and whole plant mass, we used equations of the form:

$$Root\ TNC\ pool = a_1*(whole\ plant\ mass)*d_1 + a_2*(whole\ plant\ mass)*d_2,$$

where a_1 and a_2 are the estimated parameters for each light or nutrient level, and d_1 and d_2 are dummy variables that take on values of 0 or 1, depending on the treatment level. Equations were fitted using the Gauss-Newton method in the non-linear procedure of Systat (SPSS Corp, Chicago, IL, USA). We assumed a normal error distribution, testing this assumption with probability plots and G-tests. We calculated Akaike Information Criteria (AIC) with a correction for small sample size (AIC_c, Hurvich and Tsai 1989, Burnham and Anderson 2002). The AIC (and AIC_c) is an estimate of the expected, relative distance between the fitted model and the true, unknown mechanism that generated the data (Burnham and Anderson 2002) and, unlike likelihood ratio tests (LRTs), can be used for selection among non-nested models. Unlike LRTs, because the use of AIC_c for model selection is not a test of significance but a selection among candidate models for the best approximating model closest to the 'truth', no significance values are associated with model selections based on AIC_c. The model with the lowest AIC_c is chosen as the best approximating model from the set of candidate models for each relationship. In general, a difference of more than 2 units in the AIC_c of two models indicates poorer support for one model over the other (Burnham and Anderson 2002). To determine treatment effects, the AIC_c for models incorporating treatment effects were compared to models not including treatment effects. We also analyzed root TNC concentrations at the final harvest using general linear models and ANOVA. We used whole plant mass as a covariate, checking that the assumption of homogeneity of slopes was valid by verifying that the interaction between whole plant mass and treatment was non-significant.

We characterized species' allocations to nutrient harvesting structures (foraging) as the functional relationships between fine root surface area versus root mass and structural root mass. In contrast to specific root area (i.e. fine root surface area / total root mass), analyzing these data as functional relationships can account for ontogenetic effects and avoids problems inherent in analyzing ratios (Jasienski and Bazzaz 1999, MacFarlane and Kobe, in review). We examined fine root area with respect to both total root mass and structural root mass. We used maximum likelihood methods to estimate parameters for these relationships, using AIC_c to choose the best approximating model from a set of candidate models. A similar set of models to those presented in Table 2.3 were used to test for these relationships. However, we reasoned that seedlings would allocate to nutrient uptake even in their establishment phase and had no *a priori* reason to expect lag phases such as in the sigmoidal curve. We therefore tested only situations where the rate of conversion of mass to area remained constant over root structural mass (or root mass; model 1); where conversion efficiency increased exponentially with root structural mass (or root mass; model 2); and where conversion followed the power law (models 3). Light and N effects were tested as described for root TNC and structural pools.

Results

Allocation to roots

The proportion of whole plant mass allocated to roots (root mass fraction, RMF) increased with an increase in light in all species (Figure 2.1) except in black oak under high N levels (Fisher's LSD, $p<0.05$). Similarly, RMF decreased with an increase in N levels except for red oak, white oak and black oak in low and high light, and red maple in low light.

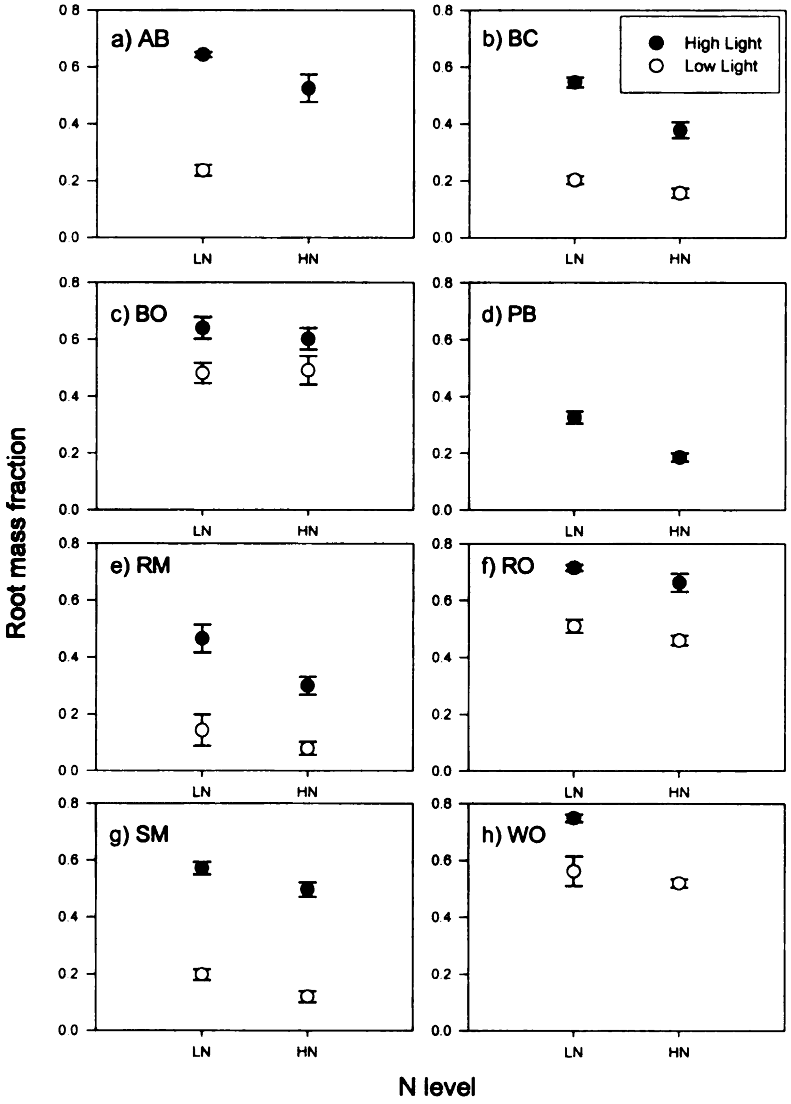

Figure 2.1 Root mass fraction of species and treatments at final harvest.
LN Low N; HN High N.

Storage of nonstructural carbohydrates

Across all species, root total nonstructural carbohydrate (TNC) pools increased with whole plant mass (Figure 2.2). At final harvest, across all species and treatments, stored reserves accounted for about 25% of root dry mass. However, there was considerable intra- and inter-specific variation so that root TNC reserves ranged from 8% to 45% of total root dry mass (Table 2.3). Because size was used as a variable in our models, we first tested for the effects of size-independent ontogenetic effects on root TNC pools (and also root surface area). Within species and treatments, harvest date did not have an effect on TNC pools or on root surface area that were independent of plant mass except in American beech where storage pools and time of harvest had significant interactions (ANCOVA, $\alpha = 0.05$; data not shown) so that results for beech need to interpreted with the caveat that the nature of the relationship between root TNC and whole plant mass is not independent of age in this species.

As expected, in all species, TNC concentrations generally increased with light and decreased with N availability (Table 2.3). For a given whole plant mass, TNC pool sizes were significantly higher under high light availability than low light availability in all species and were higher under low N than high N in most species (AIC_c, Figure 2.2), with differences generally increasing with plant mass. In the case of red oak under high light, and black cherry under low light, however, a model that did not incorporate N effects had marginally greater support than a model with N effects ($\Delta AIC_c = 1.217$) indicating negligible N effects. Note that in all other species, ΔAIC_c, the difference between the AIC_c of a model with N (or light) effects and a model without these effects was greater than 2, indicating substantial support for N (or light) effects (Burnham and Anderson 2002).

We also examined intra- and inter-specific variation in the two components of TNC – starch and soluble sugars (e.g. glucose and sucrose). In most species-treatment combinations, starch is the dominant form of stored carbohydrate. The one exception is shade-intolerant paper birch, which has higher amounts of soluble sugars than starch (Figure 2.3), irrespective of N availability. Excluding paper birch, starch concentrations were between 1.5 and 8 times higher than soluble sugars, although in black cherry, starch concentrations were 15-30 times higher than soluble carbohydrate concentrations including sugar alcohols (Table 2.4). In most cases, differences in TNC among resource treatments were driven by changes in starch; soluble sugar concentrations were similar across treatments.

To test the assumption that most TNC is stored in roots, we also measured non-structural carbohydrates in stems in the last harvest. Although most seedlings had higher TNC pools in roots than stems, there was substantial variation among species and treatments in root versus stem allocation of TNC (Table 2.5). Red oak and white oak stored close to 90% of their reserves in their roots while red maple stored approximately 55 – 60% in roots. In most species, the proportion of TNC stored in roots did not vary significantly with treatment except for red oak and sugar maple. In red oak, the proportion of (root+stem) TNC that is in roots, adjusted for whole plant mass, decreased with light availability but not N levels (ANCOVA, $p < 0.05$). In sugar maple, this proportion declined by more than half when both light and N levels were reduced (ANCOVA, $p < 0.05$).

Table 2.3 Mean root TNC concentration (mg g^{-1}) ± S.E., across species and treatments in seedlings at the final harvest. Means followed by different letters are significantly different. Means comparison with Bonferroni corrections ($\alpha = 0.05$). Treatment abbreviations are: HLHN High light, high N; HLLN High light, low N; LLHN Low light, high light; LLLN Low light, low N.

	HLHN	HLLN	LLHN	LLLN
AB	361.4 ± 45.6^{a}	386.3 ± 20.2^{a}	-	175.3 ± 27.9^{b}
BC	78.8 ± 28.6^{a}	133.5 ± 13.6^{a}	4.6^{b}	4.8^{b}
BO	332.9 ± 19.2^{a}	359.1 ± 20.5^{a}	198.1 ± 33.8^{b}	294.3 ± 42.9^{ab}
PB	100.4 ± 7.2*	89.2 ± 14.4*	-	-
RM	180.3 ± 14.0^{a}	247.9 ± 13.9^{a}	-	-
RO	328.9 ± 49.7^{a}	386.0 ± 18.7^{a}	238.2 ± 21.4^{b}	286.7 ± 17.8^{b}
SM	202.2 ± 18.5^{a}	253.3 ± 14.5^{a}	75.2^{b}	-
WO	368.8 ± 22.8^{ab}	449.8 ± 17.4^{a}	329.5 ± 28.0^{b}	326.2± 35.3^{b}

* Could not test for mean differences since whole plant biomass was a significant covariate.

Table 2.4 Mean concentration (mg g^{-1}) ± S.E. of sugar alcohols, total soluble carbohydrates and starch in black cherry seedlings under high light at the final harvest. Treatment abbreviations are explained in Table 2.3 legend.

Treatment	Sorbitol	Myoinositol	Soluble carbohydrates	Starch
HLHN	1.43 ± 0.2	0.22 ± 0.1	4.92 ± 1.0	73.91 ± 27.7
HLLN	1.41 ± 0.1	0.24 ± 0.1	3.92 ± 0.4	129.57 ± 13.6

Table 2.5 Mean proportion of total TNC found in roots ± SE, across species and treatments in seedlings 105-106 days old. Treatment abbreviations are explained in Table 2.3 legend.

	HLHN	HLLN	LLHN	LLLN
AB	0.75 ± 0.04	0.83 ±0.01		
BO	0.89 ±0.02	0.89 ± 0.02	0.77 ± 0.02	0.80 ± 0.02
RM	0.62 ±0.03	0.54 ± 0.06		
RO	0.88 ±0.03^{ab}	0.94 ± 0.00^{a}	0.84 ± 0.02^{b}	0.88 ± 0.02^{b}
SM	0.77 ±0.03^{a}	0.76 ±0.04^{a}		0.35 ± 0.02^{b}
WO		0.95 ± 0.00	0.87 ± 0.01	0.87 ± 0.04

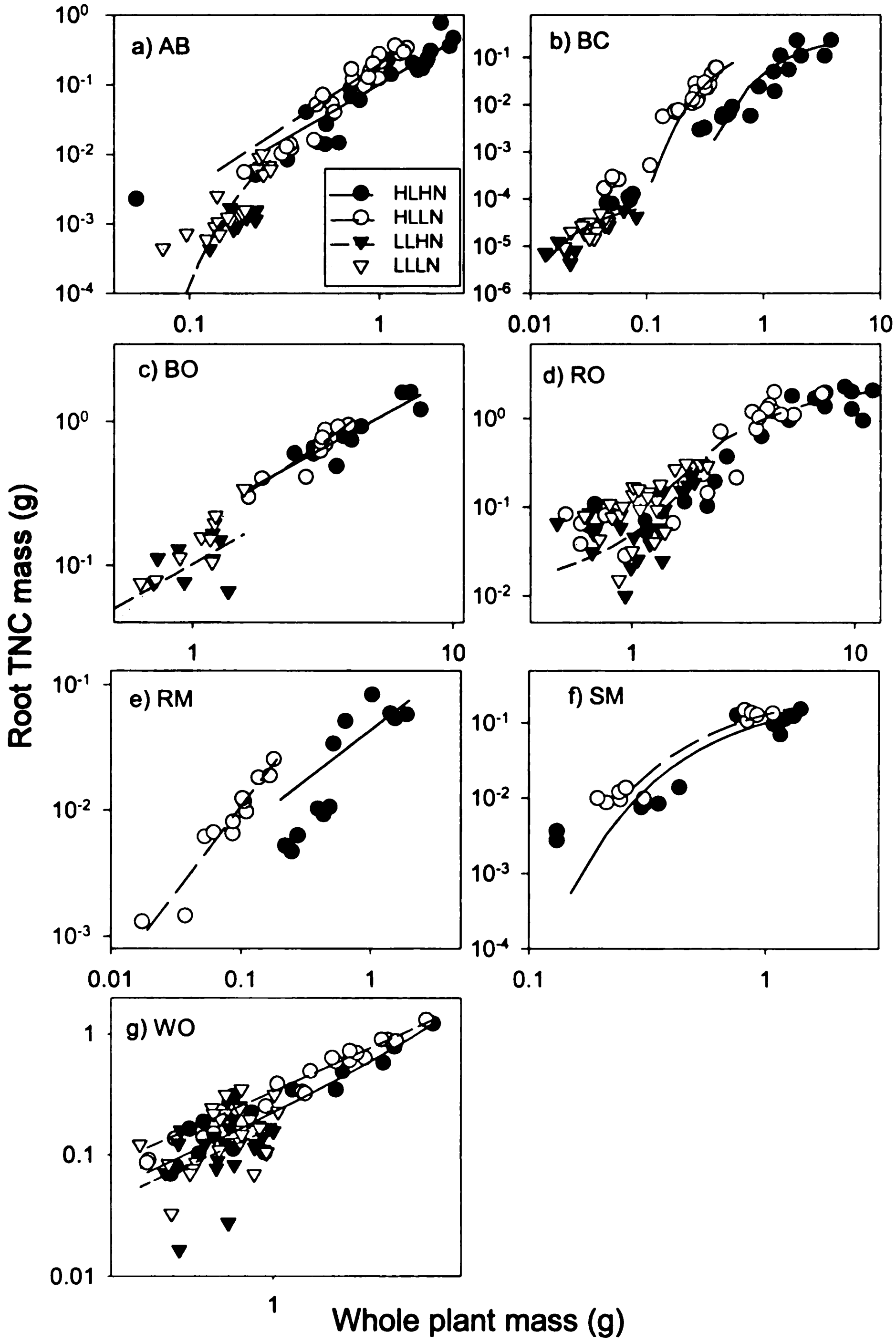

Figure 2.2 Root TNC pools (g) as a function of whole plant mass (g). Note that sugar maple and red maple have data for high light only. Lines represent best-fit equations. Treatment abbreviations are explained in Table 2.3 legend.

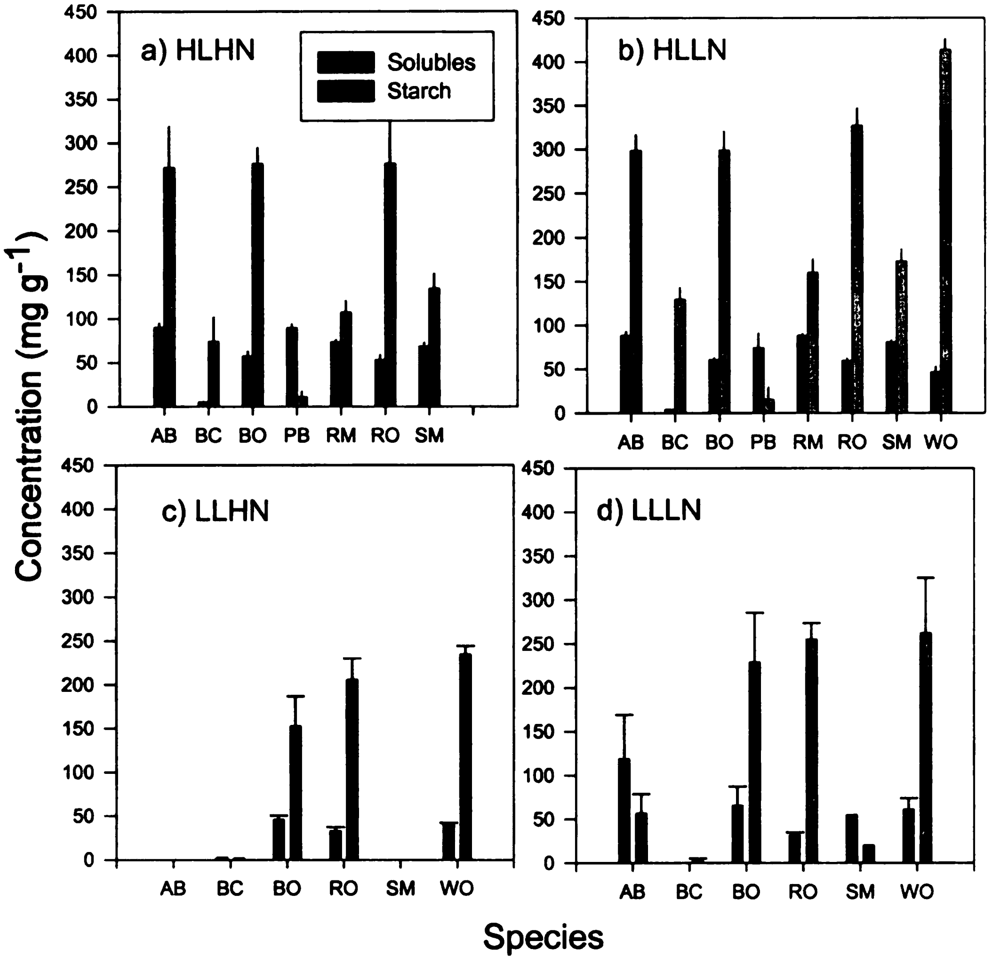

Figure 2.3 Soluble sugars and starch concentrations in roots at final harvest. Treatment abbreviations are explained in Table 2.3 legend.

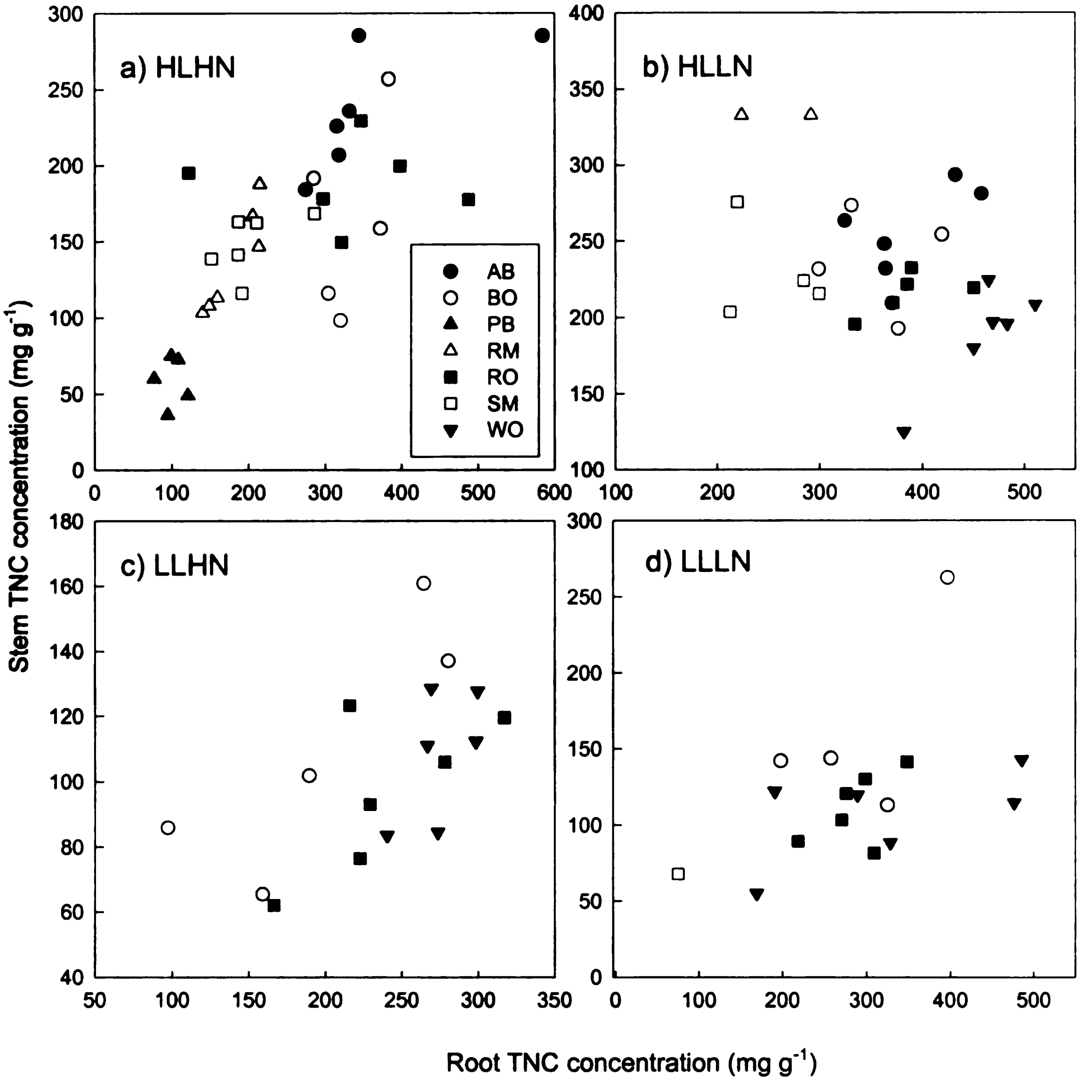

Figure 2.4 Stem and root TNC concentrations in species at final harvest. Treatment abbreviations are explained in Table 2.3 legend.

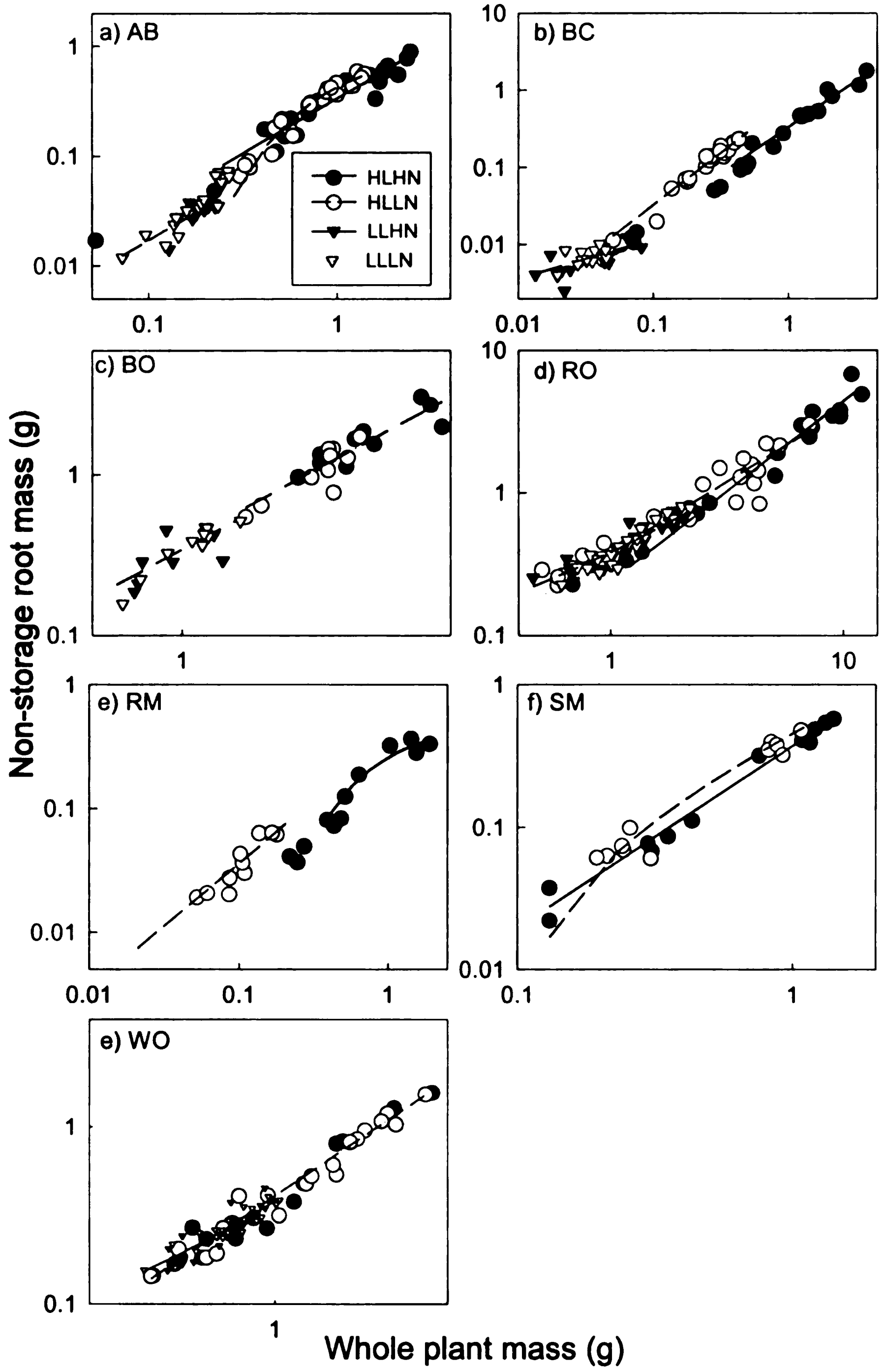

Figure 2.5 Non-storage root mass as a function of whole plant mass. Note that red maple and sugar maple have data for high light only. Lines represent best-fit equations. Treatment abbreviations are explained in Table 2.3 legend.

Changes in TNC pools as reported above are the result of changes in both mass allocation between stems and roots and the concentration of labile carbohydrates in those organs. In red oak under high light and high N, for instance, root TNC concentration is approximately double stem TNC concentrations (Figure 2.4), while RMF and stem mass fraction (SMF) are ~ 0.65 (Figure 2.1) and 0.2 respectively. The high proportion of TNC stored in roots in this species, is hence due to both the higher concentrations in roots and the higher root mass fraction. Similarly, the reduction in root TNC pools under low light and N levels in sugar maple is due not only to the decrease in root TNC concentrations by approximately half (Figure 2.4), but also because RMF decreases from 0.5- 0.6 under high light to 0.2 under low light and low N (Figure 2.1) while SMF increases from ~0.2 to ~0.4 (data not shown).

Total stored reserves in our study species varied more than tenfold, from a low of ~3% of dry mass in paper birch under high light and N availability to ~36% of dry mass in white oak under high light and low N. As in the case of root TNC concentrations, whole plant TNC concentration also increased with increasing light availability and decreasing N availability.

Non-storage mass in roots

In all species, non-storage mass increased with whole plant mass (Figure 2.5). Similar to root TNC-plant mass relations, structural root mass-plant mass relations varied with N and light treatments, but responses were weaker and seen in fewer species. Under low light, only red oak and American beech showed N treatment effects, both showing weak decreases in structural root mass with increases in N (Figure 2.5). Under high light, American beech, black cherry, red maple, and sugar maple decreased non-storage root mass in response to higher N. Red oak models of non-storage root mass as a function of whole plant mass were different between N treatments (AIC, Figure 2.5) under both high and low light. However, especially under low light, support for a model with N effects was marginal: ΔAIC between models with and without N effects was only 0.6, indicating that little information is lost if N effects are not specified. Under high light, the difference between high and low N arises from higher variance estimates under high N (0.513) versus low N (0.280) and not the parameter estimates governing the functional relationship between root structural and total plant mass. In black oak and white oak, even though root TNC pools decreased with increased N, N did not influence structural root mass, regardless of light availability (Figure 2.5).

Surface area of fine roots

In high light, for a given structural root mass, American beech, black oak, red oak and sugar maple had greater fine root surface area under low N than high N. In contrast, red maple had higher root surface area under high than under low N (although there was limited overlap between N treatments in structural root mass). White oak was the only species in which root surface area showed no response to N (Table 2.6). In black cherry, functional relationships were very similar for high and low N although their variances were different. Under low light, only shade intolerant black oak and red oak, generally highly sensitive to light limitation, showed increased allocation to fine root surface area under low N. American beech, black cherry and white oak did not vary root surface area in response to N level under low light.

To compare our results on species foraging patterns with conventional metrics of root morphology (such as specific root area), we also analyzed fine root surface area relative to total root mass (Table 2.7). When fine root area of red oak under low light and sugar maple under high light are normalized to total root mass instead of non-storage root mass, neither showed a significant response to N level. However, both increased root surface area per unit non-storage root mass under low N. Thus, normalizing fine root area to total root mass can obscure plant responses to nutrient environments.

Table 2.5 Maximum likelihood estimates and 95% support for the parameters of models relating fine root surface area of roots (FRSA) with diameter < 1mm and structural root mass (SRM) (g). The best supporting models were selected using AIC$_c$. Treatment acronyms are explained in Table 2.3 legend.

Species	Treatment	Model	N	R^2	*A*- 95% support	*B* - 95% support
AB	HLHN	$FRSA=153.540*(SRM^{0.593})$	22	0.81	(128.80,178.28)	(0.418,0.768)
	HLLN	$FRSA=246.923*(SRM^{0.775}$	24	0.80	(186.19,307.65)	(0.537,1.012)
	LLHN, LLLN	$FRSA=285.710*SRM$	30	0.76	(259.44,311.98)	
BC	HLHN	$FRSA=324.617*SRM$	18	0.98	(303.22,346.01)	
	HLLN	$FRSA=368.757*SRM$	23	0.86	(333.22,404.30)	
	LLHN, LLLN	$FRSA=9.041*(SRM^{0.194})$	24	0.05	(-9.19,27.28)	(-0.21,0.60)
BO	HLHN	$FRSA=115.364*SRM$	10	0.34	(79.76, 150.76)	
	HLLN	$FRSA=147.240*SRM$	11	0.60	(126.55,167.93)	
	LLHN	$FRSA=87.795*(SRM^{0.546})$	10	0.63	(52.53,123.06)	(0.19,0.91)
	LLLN	$FRSA=171.290*SRM$	11	0.53	(134.07,208.513)	
RO	HLHN	$FRSA=97.939*SRM$	24	0.78	(83.002,112.877)	
	HLLN	$FRSA=150.533*SRM$	22	0.80	(130.499,170.568)	
	LLHN	$FRSA=140.516*SRM$	23	0.58	(125.260,155.772)	
	LLLN	$FRSA=201.977*(SRM^{1.350})$	24	0.78	(160.762,243.191)	(1.023,1.676)
RM	HLHN	$FRSA=228.339*(SRM^{0.594})$	18	0.96	(192.094,264.583)	(0.493,0.694)
	HLLN	$FRSA=500.823*SRM$	17	0.75	(279.036,722.609)	
SM	HLHN	$FRSA=130.256*(SRM^{0.442})$	12	0.87	(104.797,155.715)	(0.289,0.594)
	HLLN	$FRSA=500.823*SRM$	9	0.80	(117.096,307.412)	
WO	HLHN, HLLN	$FRSA=117.555*SRM$	38	0.75	(105.087,130.024)	
	LLHN, LLLN	$FRSA=125.809*SRM$	46	0.14	(111.377,140.242)	

Table 2.6 Maximum likelihood estimates and 95% support for the parameters of models relating fine root surface area of roots (FRSA) with diameter < 1mm and total root mass (TRM) (g). The best supporting models were selected using AIC$_c$. Treatment acronyms are explained in Table 2.3 legend.

Species	Treatment	Model	N	R^2	*A*- 95% support	*B* - 95% support
AB	HLHN	FRSA=122.499*(TRM$^{0.513}$)	22	0.79	(107.417,137.581)	(0.352,0.674)
	HLLN	FRSA=171.155*(TRM$^{0.662}$)	24	0.79	(144.675,197.636)	(0.453,0.870)
	LLHN, LLLN	FRSA=264.608*TRM	30	0.78	(259.44,311.98)	
BC	HLHN	FRSA=284.913*TRM	19	0.97	(262.785,307.040)	
	HLLN	FRSA=207.234*(TRM$^{0.749}$)	23	0.84	(127.891,286.578)	(0.516,0.981)
	LLHN, LLLN	FRSA=9.017*(TRM$^{0.193}$)	24	0.05	(-9.122,27.157)	(-0.207,0.594)
BO	HLHN	FRSA=76.257*TRM	11	0.37	(56.019,96.495)	
	HLLN	FRSA=92.055*TRM	11	0.57	(78.828,105.283)	
	LLHN	FRSA=74.829*(TRM$^{0.546}$)	12	0.68	(57.710,91.949)	(0.241,0.760)
	LLLN	FRSA=88.591*(TRM$^{0.538}$)	11	0.44	(54.501,122.682)	(-0.038,1.115)
RO	HLHN	FRSA=69.235*TRM	24	0.78	(58.973,79.498)	
	HLLN	FRSA=90.221*TRM	22	0.81	(78.402,102.040)	
	LLHN,LLLN	FRSA=117.637*TRM	48	0.62	(108.026,127.248)	
RM	HLHN	FRSA=214.214*(TRM$^{0.634}$)	18	0.96	(183.484,244.945)	(0.534,0.735)
	HLLN	FRSA=375.203*TRM	17	0.82	(313.065,437.342)	
SM	HLHN, HLLN	FRSA=127.218(TRM$^{0.442}$)	21	0.77	(105.474,149.362)	(0.302,0.583)
WO	HLHN	FRSA=66.709*TRM	17	0.62	(49.801,83.617)	
	HLLN	FRSA=66.073*TRM	24	0.85	(59.860,72.286)	
	LLHN, LLLN	FRSA=48.749*(TRM$^{0.361}$)	48	0.09	(32.420,65.078)	(-0.009,0.731)

Discussion

Distinguishing storage and non-storage pools

In comparing root storage versus non-storage mass responses of species to light and N availability, changes in allocation to root mass across treatments are largely due to changes in TNC rather than in non-storage root mass. TNC levels responded to treatments in more species than non-storage mass. In species that responded to light and N with both TNC and non-storage mass, the magnitude of the TNC effect was greater. Take for example, American beech seedlings of ~1.2 g whole plant mass under high light. Under high N, RMF for seedlings of this size was ~0.48 and under low N, ~0.64. Hence, root mass of seedlings weighing 1.2g in each treatment was 0.576g under high N and 0.77g under low N. Using the functional relationships modeled for the change in TNC and non-storage root mass as a function of whole plant mass (Figures 2.1 and 2.2), we can calculate that under low N, ~0.28g of root mass comprised TNC and 0.49g non-storage root mass. Similarly, under high N, TNC constituted 0.135g of root mass and the remainder, 0.441g, non-storage root mass. Thus, although at first glance, the higher RMF under low N could be construed as evidence of allocation to maximize resource uptake, our results show that the increase in root mass is driven by the more than doubling of TNC from 0.135g under low N to 0.28g under high N. At the same time, non-storage root mass increased ~ 11% from 0.441g to 0.49g. Hence, our results suggest that the increase in root mass commonly seen as a result of low N is largely due to an increase in the storage of assimilated carbon and may not necessarily lead to increased nutrient uptake.

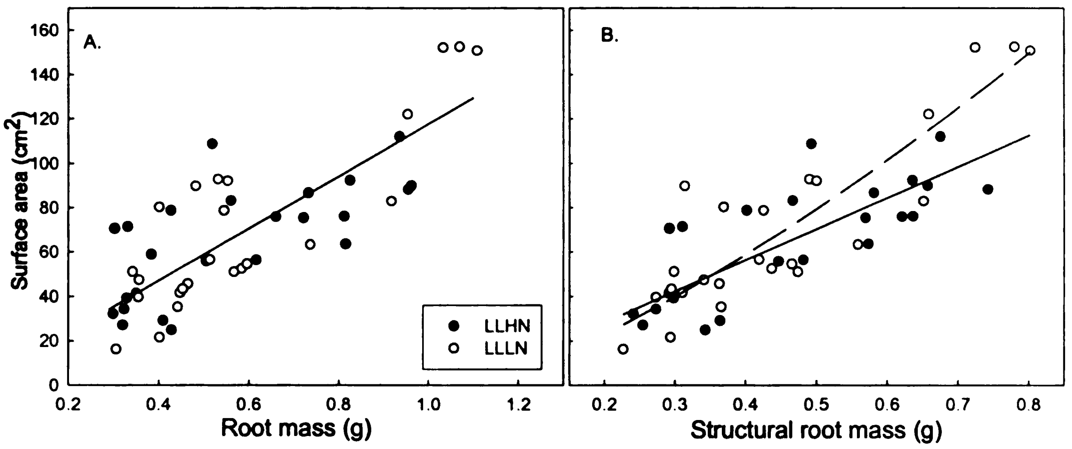

Figure 2.6 Surface area of fine roots (< 1mm diameter) as a function of A. Root mass; and B. Structural root mass under low light in red oak. Lines represent best-fit equations. Treatment abbreviations are explained in Table 2.3 legend.

Failing to differentiate between root non-storage and storage tissue also can lead to erroneous conclusions on species adaptations to nutrient environments. Fast-growing species from nutrient-rich habitats are generally thought to be characterized by allocation patterns that allow high nutrient uptake (Grime 1977). However, species comparisons of root morphology (specific root area or specific root length) are not always consistent with this theory (e.g., Poorter and Remkes 1990, Fransen et al. 1998). The inconsistency between theory and root morphology may arise because physiological plasticity in acquiring nutrients is as, or more, important as morphological plasticity (Farrar and Jones 2000). However, our results suggest that morphological characteristics normalized to root mass may mask allocation responses to variable nutrient supply. Under low N, sugar maple increased foraging under high light and red oak increased foraging even under low light, as indicated by the surface area of fine roots as a function of non-storage root mass. These results would have been obscured had we analyzed allocation to nutrient capture using a conventional metric normalized to total root mass (e.g. Figure 2.6).

Intraspecific variation in allocation to carbohydrate reserves

Although TNC responses to light availability are not entirely consistent among previous studies, our findings that TNC stores are positively correlated with light availability and negatively correlated with nitrogen availability are consistent with physiological studies and carbon-nutrient balance theory. Per theoretical expectations, an increase in the availability of carbon relative to N should result in increased carbon storage. In our study, increased availability of carbon relative to nitrogen resulted from high light and low N treatments. In genetically modified tobacco plants varying in maximum photosynthetic rates (due to variation in Rubisco expression), genotypes with higher carbon production also had higher carbon storage (Fichtner et al. 1993). Similarly, TNC concentrations increased with light availability in roots of red oak saplings (Naidu and DeLucia 1997) and European beech (Gansert and Sprick 1998). On the other hand, light had negligible effects on root TNC reserves in 2-year-old northern temperate seedlings in field conditions (Canham et al. 1999). Kobe (1997) argued from theory that opportunity costs of allocating to storage would be lower under low light, and in fact found higher root TNC concentrations under low light than under high light in his study of sugar maple and white ash saplings, but did not explicitly consider soil resource variation. Species in our study responded strongly to nitrogen availability by increasing carbohydrate stores (in particular, starch) under reduced levels of N availability, consistent with crop and grass species (Terashima and Evans 1988, Mooney et al. 1995, Stitt and Krapp 1999).

The build-up of starch under low N availability could arise from the close physiological linkage between starch and N metabolism. Under low N, increased TNC could result from N limitations to non-storage growth. However, allocation to carbon reserves can be decoupled from growth; irrespective of growth rates in genetically modified and wild type tobacco plants, high levels of nitrate in plant tissue inhibited expression of genes involved in starch synthesis, while starch accumulated under low levels of nitrate (Scheible et al. 1997). Hence under high light availability, coupled with low N levels, 'excess' carbon accumulates. Conversely, under high N, lower TNC levels result from the nitrate-induced inhibition of starch formation and the growing shoot's high demand for available carbon (Paul and Stitt 1993).

Within the high light treatment, red oak was the only species that did not have significantly higher TNC pools under low N. Although it is most likely that small sample sizes and within treatment variability obscured differences among N treatments, red oak also is tolerant of a wide range of nutrient conditions and the low N levels used in our experiment may not have limited structural growth and carbon demand. Consistent with nitrate-induced inhibition of starch formation, red oak assimilates very low levels of nitrate, regardless of N availability (Truax et al. 1994). Further, it is also possible that the large seed size of the species, the largest in our experiment, obscured treatment effects. Under low light, seedlings would have used up seed carbon stores earlier than under high light, so that differences in TNC between N levels were apparent even over the short duration of our experiment. Under high light, however, carbon was presumably not limiting, and N limitation did not manifest during our experiment due to nutrient subsidies from the seed under the low N treatment.

Although the accumulation of stored carbohydrates under limiting N has often been viewed as a "passive" process arising from resource imbalances, we argue that TNC storage also has an active component. Under conditions that presumably would be least favorable for "passive" accumulation of TNC – low light with high nitrogen – TNC concentrations still attained 20-30% of root mass for all three oak species. The only other species for which we had data from this treatment was black cherry and its TNC concentrations were negligible. Additional species were represented under low light and low N and, excluding black cherry, TNC concentrations ranged from ~ 10% in sugar maple to ~ 30% in the oak species. The physiological regulation of starch formation as discussed above and allocation to a 'baseline' level of root carbohydrate stores even when both light and N are possibly limiting suggests that TNC storage is also an important active process in these species (Chapin et al 1990).

In addition to their role in frost tolerance (Kozlowski 1992), spring regrowth (Loescher et al. 1990) and recovery from disturbance due to disease, herbivory (Kobe 1997, Marquis et al. 1997) or fire (Hoffmann et al. 2003), long-term root carbon stores are also an important source for both fine root growth and mycorrhizae (Langley et al. 2002). Mycorrhizae receive most or all their carbon from plant hosts (Treseder and Allen 2000), with root carbohydrates acting as the initial cue for infection, which once established, can in turn influence TNC concentrations. Further, exudation of soluble carbon compounds into the rhizosphere could also support fungal symbionts (Schwab et al. 1991). Indeed several studies have indicated that elevated levels of root TNC, and of soluble carbohydrates in particular, are closely correlated with mycorrhizal colonization (Same et al. 1983, Graham et al. 1997). In our study, starch was the dominant form of reserve carbohydrate in all species, as expected (Kozlowski 1992), with the exception of paper birch. In this ectomycorrhizal species (Godbold et al. 1997), soluble carbohydrates constituted the predominant form of stored carbohydrate. Further, consistent with its high root concentrations of soluble sugars, this shade-intolerant, fast-growing species also has high rates of rhizodeposition of labile carbon compounds (Bradley and Fyles 1995), thus stimulating soil microbial activity and higher rates of soil nutrient cycling (Bradley and Fyles 1995), which likely enable the high nutrient uptake rates seen in this species (Bradley and Fyles 1995).

Conclusions

The high levels of TNC we found in roots highlight the importance of taking this carbon pool into account when investigating biomass allocation in response to variation in above and below ground resource availability. Changes in root mass allocation with nutrient and light levels are driven largely by changes in TNC pools in roots, and to a lesser extent by changes in non-storage tissue to allow the acquisition of additional resources. However, in most species, the conversion of non-storage tissue to surface root area is more efficient under low N. Furthermore, since storage and non-storage pools respond independently to light and nutrient levels, neglecting the distinction between allocation to these two functionally distinct pools can potentially lead to errors in interpreting species responses to soil resource environments. Moreover, allocation to either pool could have different consequences on how the species performs under different resource environments with storage likely favoring long-term survival and allocation to structure favoring short-term growth (e.g. Kobe 1997). Thus, distinguishing between pools of structure and storage is critical from methodological, conceptual and functional perspectives.

Acknowledgements

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Chapter 3

Nonstructural carbohydrate storage underlies species trade-off between growth and survival in seedlings of northern temperate forest trees

Introduction

Interspecific differences in tolerance of low resource environments influence species composition in those environments. For example, differences in the ability of tree species to survive in low light environments are important determinants of successional dynamics in temperate forests (Kobe et al. 1995). Species also vary widely in their tolerance of low soil resources, a factor which likely determines their distributions across landscapes differing in soil resource availability (Schreeg et al. 2005).

For trees, there is some evidence that a species' ability to survive in low resource environments is at the expense of reduced growth potential in high resource environments (for light: Kitajima 1994, Kobe et al. 1995, for soil resources: Schreeg et al. 2005). If competitive ability is positively related to growth potential in resource rich environments (Grime 1977), then the inverse relationship between species survival in low light environments and growth in high resource environments can be described as a trade-off in survival and competitive ability. Suites of traits are thought to underlie these trade-offs. For example, higher specific root surface area and specific root length are found more often in species from high soil resource than low soil resource environments when grown under the same conditions (Reich et al. 1998, Craine et al. 2001, Comas et al. 2002), although a link between growth rate, habitat and root structure is not always apparent (Poorter and Remkes 1990). Root morphological traits that promote persistence in low resource environments include longer main root axes: species from drier habitats sometimes have longer main root axes than those from wetter habitats when grown under the same conditions (Nicotra et al. 2002) although this is not always the case (Schreeg et al. 2005). Leaf traits that allow persistence in low light environments include greater leaf longevity, low respiration rates, low leaf-mass ratios, and thick, tough leaves that reduce susceptibility to herbivory. These traits may occur at a tradeoff with high photosynthetic capacity, high specific leaf area and leaf area ratio, traits that confer high growth potential in high light (Walters and Reich 1999). Survival in low light environments may also be linked with greater storage of nonstructural carbohydrates (Kobe 1997, Canham et al. 1999) which may be reflected in the lower allocation to leaves observed in temperate shade tolerant species (Walters and Reich 1999) and may come at the expense of growth rate (Chapin et al. 1990).

Although nonstructural carbohydrate stores are essential for future growth such as spring regrowth in deciduous species (Loescher et al. 1990) and for regrowth following defoliation due to disease or herbivory (Kobe 1997, Marquis et al. 1997), both theory and empirical evidence show that allocation to storage is necessarily at the expense of allocation to structure and resource-harvesting and transporting components (Chapin 1990, Kozlowski 1992). Previous research on carbohydrate storage has emphasized their importance for buffering the impacts of variable resource environments (Bloom et al. 1985). Higher levels of stored carbohydrates are also expected to occur in species subject to unpredictable disturbance events (Iwasa and Kubo 1987) including fire (Miyanishi and Kellman 1986, Hoffmann et al. 2003). Levels of stored carbohydrates also vary with shade tolerance (Kobe 1997) and likely with tolerance of low soil fertility (Steinlein et al. 1993).

I propose that allocation to carbohydrates increases survival in low-resource environments and can be manifest as either shade tolerance (Kobe 1997, DeLucia et al. 1998) or tolerance of low soil resource environments. Though roots are the primary site for storage of total nonstructural carbohydrates (TNC) (Loescher et al. 1990) accounting for as much as 40% of root mass (Nguyen et al. 1990, Kobe 1997, Canham et al. 1999, Chapter 2), root mass allocation is rarely differentiated between allocation to storage and structural tissue. I suggest that allocation to root storage versus root structural pools may be an important whole-plant physiological mechanism underlying species trade-offs between high resource growth and low resource survival.

To test these ideas, I carried out a greenhouse experiment investigating the effects of resource levels on allocation to either root storage or root structural tissue in seedlings of eight temperate forest tree species. I examined effects of light and N on allocation within these species in Chapter 2. Here, I tested the following hypotheses:

- Species with higher allocation to total nonstructural carbohydrates (TNC) will have lower growth rates.
- Species capable of persisting in low resource environments (light or nutrients) will have higher allocation to TNC than species associated with high-resource environments.
- Variation among species in root mass fraction with resource environments are largely due to changes in root TNC, rather than to changes in structural root mass.

Materials and methods

Species and seedling measurements

I selected 8 tree species for the experiment that encompass a wide range of shade tolerances and associations with soil resource levels (Table 3.1) based on both literature of northern temperate forest species in general (e.g., Burns and Honkala 1990, Abrams

2003) and on Michigan's forests in particular (e.g., Cohen 2000, Leahy and Pregitzer 2003). The species used were *Acer rubrum* L. (red maple), *A. saccharum* Marsh. (sugar maple), *Quercus velutina* Lam. (black oak), *Q. rubra* L. (red oak), *Q. alba* L.(white oak), *Prunus serotina* Ehrh. (black cherry), *Fagus grandifolia* Ehrh. (American beech) and *Betula papyrifera* Marsh. (paper birch). Seed sources, germination, greenhouse growing conditions, and measurements of seedling morphology are described in detail in Chapter 2. Briefly, I grew seedlings at two light levels (~2% and ~22% full sun) and two nitrogen levels (0.5 mg/L and 50 mg/L of N in a modified Hoagland's solution). These light levels mimic the endpoints in the range of light conditions from understorey to large tree fall gaps, encountered in northern lower Michigan (Schreeg et al., 2005). Similarly, the high N treatment approximates available nitrogen levels in high fertility moraines in the Manistee National Forest in northern lower Michigan (Zak et al. 1986, Kobe, unpublished data). I measured TNC in roots of seedlings harvested at regular intervals (Table 2.1; see Chapter 2). Briefly, the method uses hot alcohol to extract soluble sugars which are then analyzed with a phenol-sulfuric acid assay (Dubois et al. 1956). Extraction residues are enzymatically digested and analyzed with a glucose-specific colorimetric assay (Roper et al. 1998).

TNC concentration was calculated as the sum of glucose equivalents of the soluble sugars and starch measured in each sample. I also calculated pool sizes (concentration x root mass) of stored carbohydrates; while concentration is a good measure of allocation to storage, the latter is a better measure, on a whole-plant basis, of available reserves (Chapin et al. 1990). I did not analyze carbohydrate levels in leaves because I was interested in stores rather than diurnal pools of sugars (Schnyder 1993).

For some species such as black cherry and sugar maple, individuals under low light were too small for TNC analysis. Individuals in these treatments were therefore composited to obtain one or more samples with sufficient mass for analysis. No paper birch seedlings under low light were available for analysis due to high mortality.

Data analysis

Mean growth rates by species within each treatment were calculated using the formula $Mass_t = Mass_0(1+r)^t$ where $Mass_t$ is the seedling whole plant mass at time t (in days), $Mass_0$ is the initial whole plant mass and r is the growth rate. This equation is equivalent to the discrete version of a compound interest formula. Species growth rates were calculated only for those species that had a minimum of three harvests.

I analyzed allocation of root mass to storage or non-storage pools by first developing a set of candidate models to separately characterize the relationships between whole plant mass and each of the two pools of root TNC and non-storage root mass (= total root mass – TNC pool) under different light and N regimes (Table 2.2). I used a linear model with no constant (Model 1) to characterize a constant allocation of whole plant mass to root storage (or non-storage). Model 2 represents an exponential increase in allocation to storage and non-storage with an increase in whole plant mass. Model 3 is the commonly used allometric scaling model (Niklas and Enquist 2001), where $b > 1$ implies allocation to root TNC (or non-storage) increases disproportionately with whole plant mass while $b < 1$ less than proportionate increases with plant size. Model 4, a sigmoidal curve, characterizes a lag in allocation to storage (or non-storage) under small sizes with increased allocation under large sizes, as has been shown under low light in some species (e.g., Kabeya and Sakai 2003). The *a* parameter represents the asymptotic TNC (or structural) pool over the range of whole plant mass in our experiment (or structural root mass) while *b* represents the rate of increase of TNC (or structural root mass) with whole plant mass. Model parameters were estimated using maximum likelihood methods (Hilborn and Mangel 1997).

I tested effects of light and N by using dummy variables. For example, for a linear relationship between root TNC pool and whole plant mass, I used equations of the form:

$$Root\ TNC\ pool = a_1 *(whole\ plant\ mass) * d_1 + a_2 *(whole\ plant\ mass) * d_2,$$

where a_1 and a_2 are the estimated parameters for each light or nutrient level, and d_1 and d_2 are dummy variables that take on values of 0 or 1, depending on the treatment level. Equations were fitted using the Gauss-Newton method in the non-linear procedure of Systat (SPSS Corp, Chicago, IL, USA). I assumed a normal error distribution, testing this assumption with probability plots and G-tests. I calculated Akaike Information Criteria (AIC) with a correction for sample size (AIC_c, Hurvich and Tsai 1989, Burnham and Anderson 2002). The model with the lowest AIC_c was chosen as the best approximating model from the set of candidate models for each relationship. The AIC, and hence the AIC_c, is not a test of significance but provides a method to choose the best possible model from a set of candidate models (Burnham and Anderson 2002). Hence no significance values are reported for model selections using AIC_c. In general, a difference of more than 2 units in the AIC_c of two models indicates poorer support for one model over the other (Burnham and Anderson 2002). To determine treatment effects, the AIC_c for models incorporating treatment effects were compared to models not including treatment effects.

To test for species differences, for every treatment, I first developed a global model for the relationship of interest (e.g., root structural mass as a function of whole plant mass) using data from all species, across all harvests. I then compared AIC_c values of species specific models with species-general models to determine if there were species effects in that treatment. I mapped uncertainty in parameter estimates (95% support) to uncertainty in functional relationships to conservatively assess species differences as regions where 95% support for the functional relationships do not overlap (Austin and Hux 2002). I also analyzed root TNC concentrations at the final harvest using ANCOVA, with whole plant mass as a covariate.

I characterized species' allocations to nutrient harvesting structures as the functional relationships between fine root surface area and root mass or structural root mass. Analyzing these data as functional relationships, in contrast to specific root area (i.e. fine root surface area / total root mass), can account for ontogenetic effects and avoids problems inherent in analyzing ratios (Jasienski and Bazzaz 1999, MacFarlane and Kobe, in review). I also examined fine root area with respect to both total root mass and structural root mass. I used maximum likelihood methods to estimate parameters for these relationships, using AIC_c to choose the best approximating model from a set of candidate models. A similar set of models to those presented in Table 2 were used to test for these relationships. However, because I had no *a priori* reason to expect lag phases in allocation to nutrient uptake in the establishing seedlings, only the first three models in Table 2.2 were tested. Light and N effects were tested as described for root TNC and structural pools.

Results

Storage of nonstructural carbohydrates

Across species and treatments, stored reserves accounted for about 25% of root dry mass at final harvest. However, there was considerable intra- and inter-specific variation, with TNC ranging from 8% to 45% of total root dry mass (Table 3.1). Chapter 2 examines effects of light and N on allocation within species. Here I use the same data to compare allocation among species. As expected, in all species, TNC concentrations generally increased with light and decreased with N availability (Table 3.1). Among species, white oak consistently had the highest TNC, followed by American beech, red oak and black oak. Under high light, black cherry and paper birch had the lowest TNC concentrations under both N levels (Table 3.1, ANOVA, $p < 0.05$) while under low light, black cherry TNC concentrations were at least an order of magnitude lower than other species.

Table 3.1 Mean root TNC concentration (mg g^{-1}) ± S.E., across species and treatments in seedlings at the final harvest. Means followed by different letters are significantly different. Means comparison with Bonferroni corrections ($\alpha = 0.05$). Treatment abbreviations are: HLHN high light, high N; HLLN high light, low N; LLHN low light, high light; LLLN low light, low N. Species abbreviations are: AB American beech; BC black cherry; BO black oak; PB paper birch; RM red maple; RO red oak; SM sugar maple; WO white oak.

	HLHN	HLLN	LLHN	LLLN
AB	361.4 ± 45.6^{a}	386.3 ± 20.2^{a}	-	175.3 ± 27.9^{b}
BC	78.8 ± 28.6^{a}	133.5 ± 13.6^{a}	4.6^{b}	4.8^{b}
BO	332.9 ± 19.2^{a}	359.1 ± 20.5^{a}	198.1 ± 33.8^{b}	294.3 ± 42.9^{ab}
PB	100.4 ± 7.2*	89.2 ± 14.4*	-	-
RM	180.3 ± 14.0^{a}	247.9 ± 13.9^{a}	-	-
RO	328.9 ± 49.7^{a}	386.0 ± 18.7^{a}	238.2 ± 21.4^{b}	286.7 ± 17.8^{b}
SM	202.2 ± 18.5^{a}	253.3 ± 14.5^{a}	75.2^{b}	-
WO	368.8 ± 22.8^{ab}	449.8 ± 17.4^{a}	329.5 ± 28.0^{b}	326.2± 35.3^{b}

* Could not test for mean differences since whole plant biomass was a significant covariate.

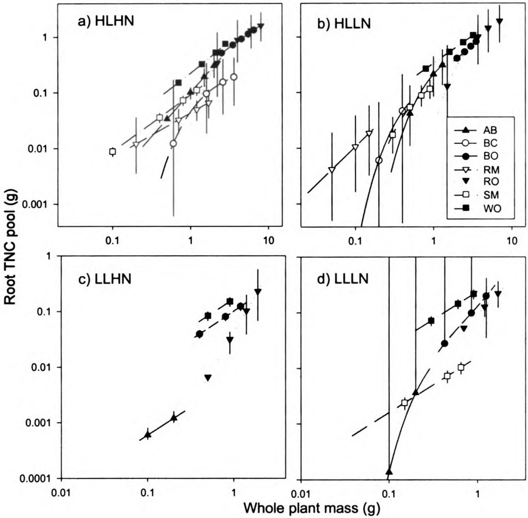

Figure 3.1 Variation in root TNC pools with whole plant mass under different light and N levels. Treatment and species abbreviations are as in Table 3.1.

Across species and treatments, the oaks consistently had the greatest pools of root TNC at any given whole plant mass (Figure 3.1). Under high light, red oak exhibited a high degree of individual variability in TNC so that patterns of storage in this species were not significantly different from any other species. Under high light and low N, root TNC was higher in white oak than in black oak and sugar maple. Under low light and low N, red oak had lower storage than white oak. The species with the slowest relative growth rate in our study, white oak, had the largest reserves compared to all other species (except red oak) under most resource conditions. White oak had higher allocation to root storage than black oak under all treatments except under low light and N, where their TNC stores were similar. When both light and N were high, American beech had significantly lower TNC at lower whole plant mass, but levels were not different from white oak when whole plant mass was greater than approximately 2g. Under high light and low N, both species had similar TNC storage across the range of whole plant mass. Under high light, regardless of N level, sugar maple had higher carbon stores than red maple. TNC stores in black cherry were similar to the maples (Figure 3.1).

White oak and black oak both showed linear or near linear increases in root TNC in all treatments except low light and low N, where black oak had an exponential increase in root TNC allocation (Appendix 1). The allometric scaling function (Model 3) provided best fits for red maple under high light: the species had less than proportional increases in root TNC under low N as total plant mass increased, and more than proportional increase in root TNC under high N. Under high light, allocation patterns in red oak, sugar maple and black cherry were best described by Model 4, as root TNC increased sigmoidally with total plant mass. However, unlike red oak, sugar maple and black cherry both approached asymptotic allocation to TNC pools under high light.

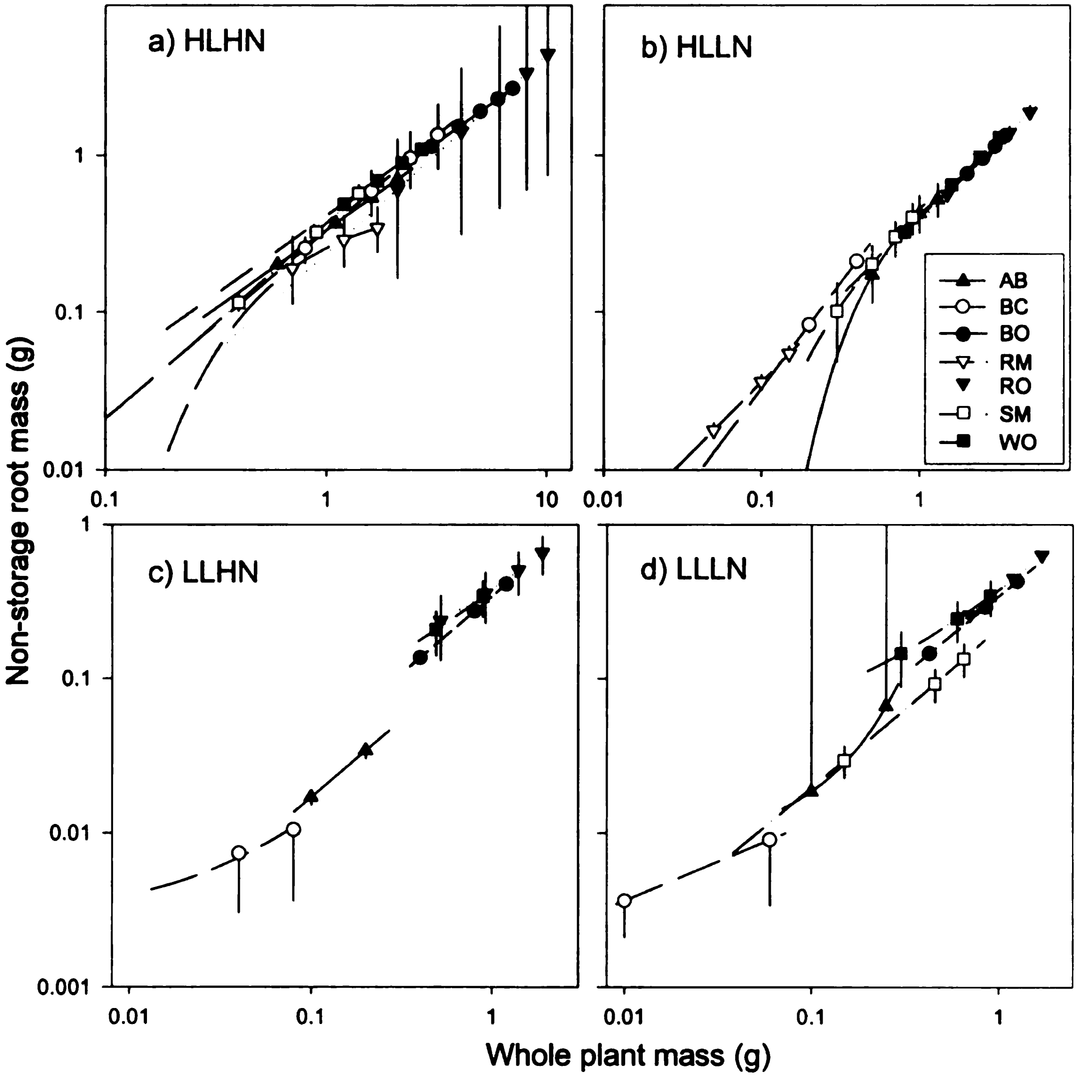

Figure 3.2 Variation in root structural pools with whole plant mass under different light and N levels. Treatment and species abbreviations are as in Table 3.1.

Non-TNC root mass

Species differences in mass allocation to roots were due largely to differences in root storage (Figure 3.1), and less so to differences in non-TNC root mass (Figure 3.2). In contrast to root storage pools, allocation to structural pools was strikingly similar across species in most treatments. Under all treatments, most species exhibited linear or near-linear responses in allocation to structural root mass with similar slopes in the relationship (Appendix 2). Red maple was the only species to exhibit asymptotic allocation to structural root mass, and this was only in high light and high N, when individuals achieved largest size. Under high light, low N, the structural root mass-whole plant mass relationship for red maple is also near-linear (Figure 3.2a and 3.2b, Appendix 2). Under high light, regardless of N level, sugar maple, red maple, black cherry and American beech did not differ from each other or the oaks in their allocation to structural root mass at a given whole plant mass (Figure 3.2a and 3.2b). Under low light, when N levels were high, American beech and black cherry had similar allocation to structural mass for a given whole plant mass though the lack of overlap in their ranges did not allow a comparison with the oaks (Figure 3.2c). When both light and N levels were low, for a given whole plant mass, allocation to structural root mass was lower in sugar maple than in the oaks (Figure 3.2d).

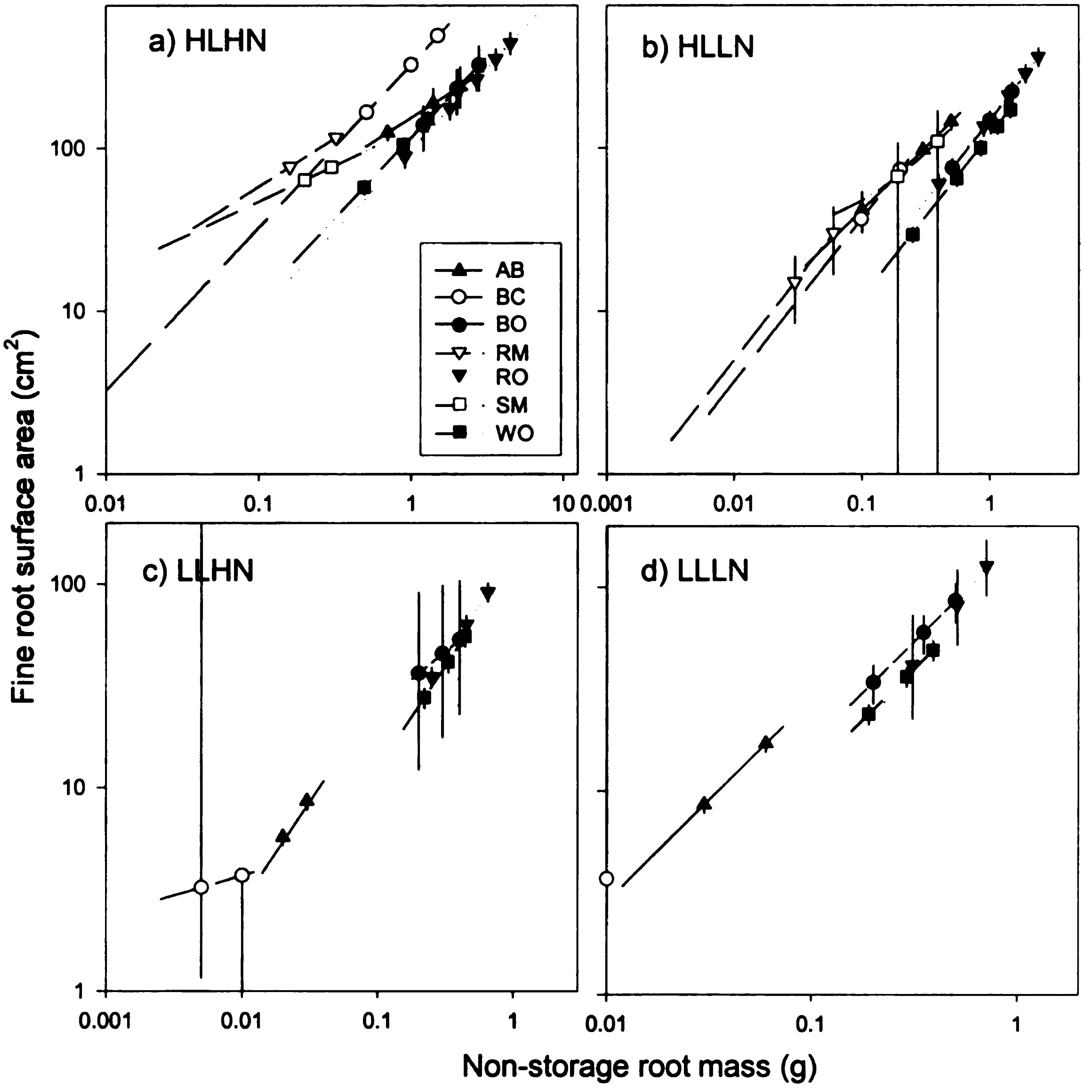

Figure 3.3 Fine root surface area as a function of structural root mass under different light and N levels. Treatment abbreviations are as in Table 3.1.

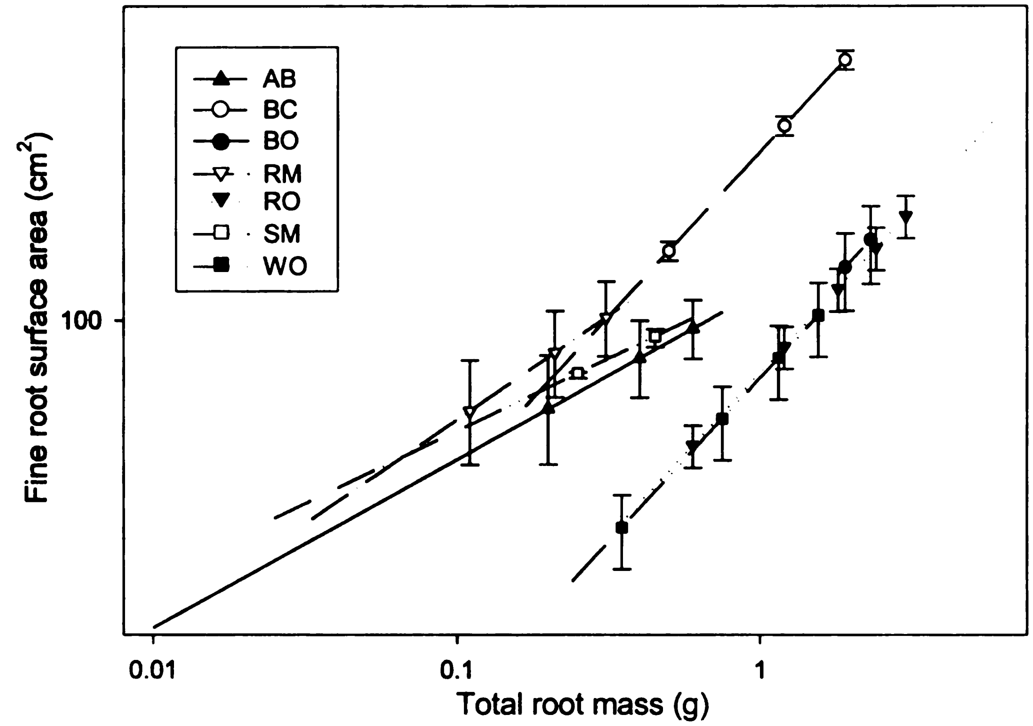

Figure 3.4 Fine root surface area as a function of total root mass in the HLHN treatment. Treatment and species abbreviations are as in Table 3.1.

Surface area of fine roots

All species had higher root surface area per unit structural mass under low N levels and higher light levels (Figure 3.3, Appendix 3). Across all treatments, the oaks had similar fine root area per unit structural root mass, which usually was lower than that of other species. When both light and N levels were high, the fast-growing shade-intolerants black cherry and red maple both had higher fine root surface area per unit of structural mass, than did the oaks, American beech and sugar maple. Sugar maple, though lower than red maple in its fine root area per unit structural root mass had higher root area per unit structural mass than the oaks. When light levels were high but N low, American beech and black cherry both had higher fine root surface area per unit structural root mass than the oaks. Sugar maple had non-significantly higher root area per unit structural mass than the oaks. Fine root surface area per unit structural mass was lower in white oak than red and black oak, but non-significantly.

To compare our results with conventional metrics of root morphology, I also analyzed fine root surface area as a function of total root mass. For the high light, high nutrient treatment (Appendix 4), American beech has root morphology similar to fast-growing red maple, black cherry and sugar maple, and higher than the oaks (Figure 3.4). Allocation patterns under other treatments are similar to those seen when analyzed with structural root mass (Appendices 3 and 4).

Relationships among TNC, growth, and survival

Within each treatment, there was an interspecific trade-off between mean growth rates and mean root TNC concentrations at the final harvest (Figure 3.5): species with high growth rates allocated smaller proportions of their root mass to carbon storage while species with large energy reserves accumulated these at the expense of growth rates. For example, in all treatments, white oak consistently had the lowest growth rate of all species but the highest allocation to root TNC. In contrast, red maple and black cherry had the highest rate of biomass accumulation under high light and high N, but the lowest root TNC levels. In all other treatments, black cherry had the highest growth rates and the lowest root TNC levels.

Although our experiment did not measure survival, I used survivorship data from an experiment that was conducted at Manistee National Forest (MNF) in northern lower

Michigan (Kobe, Kunkle and Walters, in prep.) to test the relationship between TNC measured in this study and survival. Data on survival of field seedlings from July 2001 to October 2002 are for seedlings grown in light levels of ~2 – 45% sun in the high fertility moraines and for 14-27% full sun in the low fertility outwash plains (Kobe, Kunkle and Walters, in prep.). Across species, field seedling survival in the moraine is positively correlated with mean root TNC levels under high light, high N (Figure 3.6a; $r^2 = 0.98$, $p < 0.01$) and low light, high N levels (data not shown; $r^2 = 0.86$, $p < 0.05$). Field survival in the outwash is positively correlated with mean root TNC levels in the high light, low N treatment (Figure 3.6b; $r^2 = 0.69$, $p < 0.05$). Fast-growing, shade-intolerant black cherry had the lowest survival in both moraine and outwash and had the lowest TNC storage under high light under both high and low N, and under low light and low N. The slow-growing shade-intolerant white oak had the highest survival on the fertile moraine and the highest storage under comparable resource levels in the greenhouse. In the low-fertility outwash plain, red oak had the lowest mortality; under comparable resource levels in the greenhouse, red oak had the second-highest storage of all species. Black oak had the second highest survival rate on the outwash plain and had TNC levels a little lower than red oak. The shade-intolerant fast growing red maple had lower TNC and lower survival than its more shade-tolerant, slower growing congener on both the moraine and outwash.

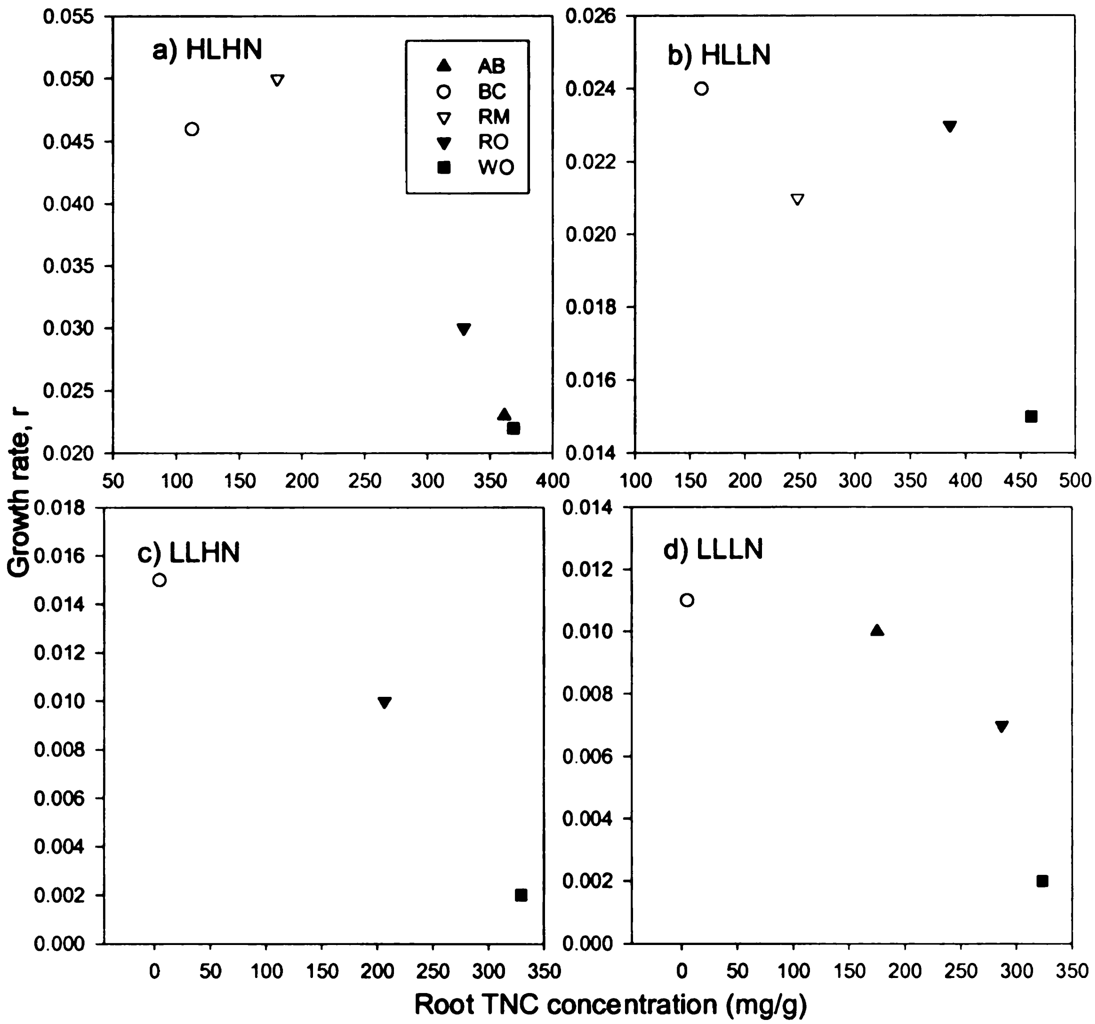

Figure 3.5 Trade-off between growth rate and root TNC concentrations under different light and N levels. Treatment and species abbreviations are as in Table 3.1.

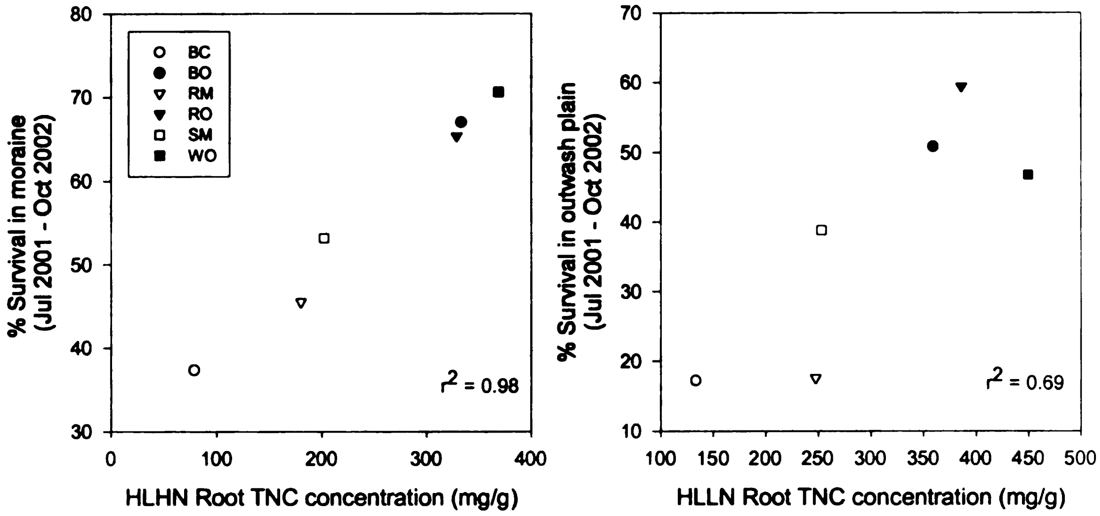

Figure 3.6 Species survival in field sites in Manistee National Forest as a function of root TNC concentrations in the greenhouse. Treatment abbreviations are as in Table 3.1

Discussion

Nonstructural carbohydrate storage, growth and survival

Our results show a strong negative relationship between TNC reserves and growth and a strong positive relationship between allocation to TNC and survival, suggesting that allocation to carbon storage likely underlies the trade-off between high resource growth and low-resource survival observed in the field (Kobe et al. 1995, Lin et al. 2002, Schreeg et al.2005). Across all treatments, species growth rates were strongly negatively correlated with allocation to TNC with slow-growing species associated with infertile sites, such as white oak and black oak, consistently having higher allocation to TNC than fast-growing species such as red maple. Our results highlight that allocation of carbon to storage implies relatively less carbon for growth (i.e., new leaves and structures for acquiring further resources). Such reserve formation at the expense of growth has been shown for some species such as sugarbeet (reviewed in Chapin 1990) and has been suggested in some tree species (Waring and Pitman 1985, Kobe 1997).

The inverse relationship between TNC and growth rates could also explain the finding that species growth rates and root tissue density are negatively correlated (Ryser 1998). Given the high specific gravity of starch (Neuhaus and Schulte 1996), a primary constituent of TNC, our results suggest that differences in starch might underlie differences in tissue density seen across species from different habitats.

Across species, there was a strong positive correlation between the proportions of root TNC and survival in the field, consistent with earlier studies that linked TNC and survival (Kobe 1997, Canham et al. 1999). Fast-growing species such as black cherry and red maple had low root carbon reserves and high mortality in the field while slow-growing species generally associated with infertile sites had high TNC reserves and had high survival in both fertile and infertile sites. Large TNC stores could enhance survival by allowing respiration when maintaining a positive carbon balance is otherwise difficult, such as under seasonal drought. TNC stores are also essential for overwinter survival, frost tolerance and for spring reflush (Loescher et al. 1990, Kozlowski 1992). Increased storage can also enhance seedling survival by facilitating recovery after disturbances (Iwasa and Kubo 1983), and after disease (Kozlowski 1992), herbivory (Marquis et al. 1997) or fire (Hoffman et al. 2003).

The relationships between root carbon reserves, growth and survival suggest that allocation to TNC could provide a mechanistic explanation for the species trade-off often documented between growth in high-resource environments and survivorship in low-resource environments (Kitajima 1994, Kobe et al. 1995, Schreeg et al., 2005). Our results show that carbohydrate storage correlates with the ability to survive low resource environments, whether of low-light tolerance in some species, such as sugar maple, or of low-nutrient tolerance in others such as white oak and black oak. Our results are consistent with findings of previous researchers who have suggested that fast-growing species survive poorly under low light because of lower investment in storage (Kitajima 1994) and have shown a correlation between low-light survival and root carbon storage (Kobe 1997).

Species often have inversely correlated competitive abilities for different resources (Tilman 1985, Huston and Smith 1987). Thus, high TNC reserves in sugar maple may confer low-light tolerance in this species, generally considered a competitor with respect to nutrient availability (Burns and Honkala 1990). Similarly, larger root carbon stores that appear to confer tolerance of low-nutrient conditions in white oak and black oak do not manifest as low-light tolerance; instead, both black and white oak are relatively shade-intolerant (Burns and Honkala 1990, Abrams 2003). Hence, TNC storage appears to confer stress tolerance, manifesting as either low-light tolerance or low-nutrient tolerance, but not both, at least in the species I studied. Indeed, allometric constraints prevent species from adapting to conditions where both nutrient and light availability are simultaneously low (Peace and Grubb 1982) although plants that can tolerate both low light and nutrient supply may be constrained by slow growth when neither is limiting (Huston and Smith 1987).

Species variation in allocation to root storage and non-storage mass

The eight species included in this study exhibited considerable variation in the amounts of root biomass allocated to storage, ranging from 8 to 45% across species and treatments. These values are similar to those found in 2-year-old field grown seedlings in Canham et al. (1999) for sugar maple and lower for black cherry and red maple. High light root TNC levels in our species were similar to tap root TNC levels in understory red maple, sugar maple and black cherry saplings in DeLucia et al. (1998).

In contrast to storage patterns, these eight species showed remarkably few differences in allocation to structural root mass, consistent with hypothesis 3. There was extensive overlap in species' allocation to structural root mass in all treatments except when both light and N are low, where sugar maple has lower structural root mass per unit whole plant mass compared to the oaks. This lack of difference in allocation to non-storage mass coupled with the variation in allocation to storage under different resource regimes suggests that patterns relating species root mass allocation with habitats may be driven largely by differences in allocation to root carbohydrate stores, rather than by allocation to root structural mass, which would be used in resource harvesting. Similarly, intra-specific variation in root mass with changes in resource availability is also driven largely by changes in TNC, rather than by changes in structural root mass (Chapter 2).

Patterns of carbohydrate allocation could arise from phylogeny and/or seed size since the oaks and American beech, all in the family Fagaceae, often had similar patterns in their responses. However, functional forms of the root TNC-whole plant mass relationships among these four species were different. The most shade-tolerant of the four species, American beech, had more than proportional increases in root TNC with whole plant mass, intermediate tolerant red oak's showed a sigmoidal response while the least shade tolerant black oak and white oak were linear or near-linear in their responses. Differences were apparent even between black and white oak, the two species that are most similar in their habitats, both being restricted to xeric, infertile sites. Though both had relatively high allocation to TNC, white oak under most conditions allocated more to root carbon reserves than black oak, suggesting the former should have greater shade tolerance and slower growth than the latter, as is the case (this paper, Burns and Honkala 1990, Abrams 2003). Further, allocation patterns were also very different among the two other congeneric species in our study. Of the two maples, the slower-growing, shade-tolerant sugar maple had higher root reserves than its fast growing congener under high light and high N. Sugar maple also had greater structural root tissue per unit whole plant mass than red maple, although red maple was far more efficient in converting this structural tissue into surface area than was sugar maple.

My analysis of fine root surface area as a function of both structural root mass and total root mass showed that conclusions on species' foraging patterns could be erroneous if root TNC is ignored in calculating specific root area. For instance, when both light and N levels are high, using the conventional metric for root surface area (normalized to total root mass), American beech appeared to allocate far more to foraging than red oak and white oak. Yet, for a given structural root mass, all three species have similar fine root surface area, signifying their conversion efficiencies of structural root mass to surface area are, in fact, similar. Further, the difference in root surface area between the oaks and the other species appears more divergent when analyzed with respect to total root mass than with structural root mass. This difference arises because species that have high root area per total root mass tend to have low root TNC (such as black cherry and red maple) while species that have lower root area per total root mass tend to have higher TNC (such as white oak, red oak and black oak). Hence, normalized to total rather than structural root mass, species differences in fine root area appear exaggerated.

Conclusions

My results suggest that storage of nonstructural carbohydrates (i.e., TNC) confers stress tolerance, allowing species to survive in environments that inhibit plant growth, such as under low light or low nutrient availability. Increased stress tolerance as a result of TNC storage can thus manifest as either low-light tolerance or as low-nutrient tolerance. Fast-growing species associated with high-resource environments, such as paper birch, typically store low amounts of TNC, suggesting root carbohydrate storage is another trait that differentiates competitors from stress tolerators (Grime 1977). The ability to tolerate low resource environments by allocation to storage at the expense of structural growth provides a mechanistic explanation for the species trade-off between fast growth under high light versus survival in low light (Kitajima 1994, Kobe et al. 1995, Lin et al. 2002) and for the recently documented trade-off between growth under high soil resource availability and survival under low-soil resource availability (Schreeg et al., 2005). Species that allocate more to storage are less competitive in high resource environments than those that allocate more to structural growth, but they have lower mortality in low resource environments.

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Chapter 4

Niche partitioning by seedlings in a tropical dry forest: effects of light and soil resource availability

Introduction

Among the many hypotheses put forward to explain species coexistence in tropical forests, prominent is that of niche partitioning. According to this hypothesis, differences among species in resource use, coupled with spatiotemporal variation in resource availability, can promote species coexistence (Denslow 1987). Because the availability of light is a fundamental determinant of growth and survival in light limited tropical forests, the majority of studies have focused on niche partitioning across light gradients. Several have demonstrated a trade-off between growth under high light and survival under low light (Hubbell and Foster 1992, Kitajima 1994, Kobe 1999); this trade-off has been shown to facilitate tree species coexistence in temperate forests (Pacala et al. 1996).

Although evidence for niche partitioning along light gradients has been equivocal, species may simultaneously segregate along soil resource gradients. Tropical forests are highly heterogeneous in soil nutrient and water availability (Sollins 1998). Such spatial heterogeneity in soils leads to species segregation along topographical gradients (Davies 2001) and soil-based habitat specialization (Palmiotto et al. 2004) suggesting that partitioning of belowground resources likely occurs in tropical forests. In temperate forests, a species trade-off between annual growth under high soil resources and survival under low soil resources likely facilitates species coexistence at the landscape level (Schreeg et al. 2005). It is likely similar trade-offs operate in tropical forests. Additionally, niche differences could also operate on two axes simultaneously because resources allocated towards light-harvesting structures (leaves, stems) are unavailable for soil-resource capture (King 1993).

In addition to spatial heterogeneity, plants also face temporal variation in resource availability. Occasional droughts are an integral feature of some tropical rainforests (Walsh and Newbery 1999). In tropical dry and moist forests, seasonality in rainfall leads to pronounced changes in both soil nutrient (Lodge et al. 1994, Campo et al. 1998) and moisture levels, possibly with differential effects on species growth and mortality (Delissio and Primack 2003). When there is high seasonal variation in resource availability, species' trade-offs between growth and survival may also be underpinned by their ability to grow rapidly during the growing season when resources are plentiful versus surviving periods of resource limitation during seasonal drought. Among species in moist tropical forest, growth in the wet season is inversely correlated with species survival in the dry season, especially under low light (Pearson et al. 2003).

In this study, I examined growth and survival responses among five co-occurring tree species to variations in light and soil resource availability in a seasonally dry tropical forest. I worked with seedlings, at which stage habitat heterogeneity is postulated to play a more extensive role (Grubb 1977). In particular, the questions I addressed were:

1. How are seedling growth and survival influenced by variation in soil resource levels?
2. Is there a trade-off between species growth rates under high light and survival under low light consistent with their distributions across light environments?
3. How does soil resource availability affect the high light growth – low light survival trade-off?
4. Does seasonal drought have a differential effect on species growth rates and survival?

Methods

Study site and species

The study area is within the Palo Verde National Park (10°21'N, 85°21'W), Guanacaste, Costa Rica. This region is classified as tropical dry forest according to the Holdridge (1969) life-zone classification. The annual average temperature is 27.4°C and rainfall 1817 mm, with a pronounced dry season from December to May (Jimenez et al. 2001). The semi-deciduous forests in the 20,000 ha Park have approximately 65 tree, shrub and liana species per 1000 m^2 or 0.1 hectare (Gillespie 1999).

The species I selected for my study were *Astronium graveolens* Jacq., *Pachira quinata* (Jacq.) WS Alverson, *Cordia gerascanthus* L., *Dalbergia retusa* Hemsl. and *Tabebuia rosea* (Bertol.) DC. The species have some soil resource affinities (Table 4.1, Eugenio Gonzalez, pers. comm.) but all co-occur as adults in the secondary forests of the Park. I selected these species on the basis of seed availability, and to present a range of seed sizes and growth habits (Table 4.1). Relative to other species in the area, *Pachira* and *Tabebuia* are fast-growing shade-intolerant species (Kitajima 2002), *Astronium* and *Dalbergia* are slow growing late-successional species (Hartshorn 1983, Piotto et al. 2004); *Cordia* is a late-successional species (Opler et al. 1975), whose growth rate is unknown. Of these five species, *Cordia* is in danger of extinction, while *Dalbergia* and *Astronium* are threatened (Jimenez-Madrigal 1998). The study species are referred to by their genus names only throughout the remainder of the paper.

Experimental methods

Based on preliminary soil analysis in March 2000, I chose three sites that differed in soil phosphorus (P) availability to use for a transplant experiment. All sites were in secondary forest areas, within 3-6 km of each other and on Ustorthent soils. The sites had a gradient in soil fertility, and in particular soil P, with site Carreta having the lowest, Ojo de Agua intermediate and Arboleda the highest levels of soil P. Although the sites also had different levels of cation availability (Table 4.2), levels of these nutrients were generally high in all sites and hence were considered unlikely to be limiting to tree growth (Tisdale et al. 1993). Potential N mineralization rates were similar in the two low P sites and higher in the high P site (Table 4.2) but once again, were not expected to be limiting to tree growth in any site. I established 140, 70 and 156 transplant plots in Carreta, Ojo de Agua and Arboleda, respectively, stratified across natural gradients in light availability in each site. Within each site, plots also naturally varied in available soil P. Each plot measured 1.5 x 1.5m, with a minimum distance of 1 m between plots. To maintain a consistent light and soil resource environment, vegetation was cleared from the plots and resprouts cut back at intervals. To reduce vertebrate herbivory, all plots were fenced to a height of 1.25m.

Table 4.1 Families, mean seed mass and growth habits of the five study species.

Species	Family	Seed mass (mg)	Purported growth, shade-tolerance	Purported soil resource associations [d]
Astronium graveolens	Anacardiacae	22.6[a]	Slow-growing, intermediate shade-tolerant[c,d]	Intermediate fertility
Cordia gerascanthus	Boraginaceae	44.9 [b]	Intermediate shade-tolerance, late successional [b,d]	High fertility
Dalbergia retusa	Fabaceae	77.4 [a]	Slow-growing, intermediate shade-tolerant, late successional [c,e]	Intermediate-high fertility
Pachira quinata	Bombacaceae	22.4 [a]	Fast-growing, shade intolerant, early successional [c,d,f]	High fertility [f]
Tabebuia rosea	Bignoniaceae	22.6 [a]	Fast-growing, shade-intolerant, early successional [g,h]	Low fertility

Sources: *a*: this study; *b*: Opler et al. 1975; *c*: Piotto et al. 2004; *d*: Eugenio Gonzalez, pers. comm.; *e*: Blair and Perfecto 2004; *f*: Cordero et al. 2003; *g*: Kitajima 2002; *h*: Hubbell 1979.

Table 4.2 Cation availability and potential N mineralization in soils in the study sites. Letters indicate significant differences between sites (ANOVA, $p < 0.05$).

Site	Cation availability (mg kg $^{-1}$ soil)								N min-eralization (mg N/ kg soil /day)
	Zn	Mn	B	Fe	Mg	Na	Ca	K	
Carreta	4.2±0.2 ^{b}	82.3±4.0 ^{a}	2.2±0.4 ^{c}	142.1±7.7 ^{a}	848.7±22.3 ^{a}	26.3±2.2 ^{a}	6138.6±163.9 ^{c}	258.5±15.2 ^{b}	0.96±0.06 ^{b}
Ojo de Agua	3.8±0.2 ^{b}	93.7±1.6 ^{a}	4.1±0.3 ^{b}	119.0±3.5 ^{ab}	829.9±27.7 ^{a}	10.7±0.7 ^{b}	7158.2±139.2 ^{b}	105.2±12.2 ^{c}	0.88±0.07 ^{b}
Arboleda	5.2±0.4 ^{a}	55.9±4.4 ^{b}	5.6±0.4 ^{a}	121.0±5.1 ^{b}	590.3±22.5 ^{b}	12.2±0.7 ^{b}	9429±236.8 ^{a}	344.0±20.4 ^{a}	2.43±0.15 ^{a}

Table 4.3 Mean ± SE (min – max) light availability, soil phosphorus and soil moisture at the study sites.

	Carreta	Ojo de Agua	Arboleda
Light availability (% full sun)	34.1 ± 2.6	12.2 ± 0.9	11.6 ± 0.3
	(6.2 – 88.2)	(5.6 – 44.3)	(5.1 – 27.2)
Inorganic soil P (mg g $^{-1}$)	2.3 ± 0.1	9.7 ± 1.7	79.5 ± 3.9
	(0.5 – 6.3)	(3.2 – 103.0)	(7.3 – 160.3)
Soil moisture (%)	26.6 ± 0.4	31.7 ± 0.9	39.4 ± 0.8
	(18.3– 61.1)	(14.9 – 41.2)	(10.0 – 84.9)

In June-July 2000, I germinated seed of *Dalbergia, Tabebuia* and *Pachira* to use in a transplant experiment. I collected *Dalbergia and Tabebuia* seed from the vicinity and purchased *Pachira* seed from a commercial source that collected seed locally. To promote mycorrhizal inoculation, I germinated seeds in flats containing a mixture of 9:1 sand:field soil, with soil from each site contributing 1/3 of the field soil component. Each germinant was transferred to a plastic nursery bag filled with soil from the site where the seedling eventually would be transplanted. In addition, I collected new germinants (i.e., seedlings retaining cotyledons) of *Astronium* and *Cordia* from the field and planted them into bags using the same procedure as for the germinated seed. Seedlings of all species were grown at 70% full sun until they were transplanted into the experimental plots over two weeks starting 1 August 2000. Each plot had 2-4 individuals of each species, planted in randomly chosen positions of a 4x4 grid with positions separated by 50 cm. Each plot thus had 10-12 individuals, with some positions in the 4x4 grid remaining vacant.

To correlate resource availability with seedling growth and survival, I measured light, soil P and soil moisture in each transplant plot. Resource levels in each site were as in Tables 4.3. I used hemispherical canopy photographs taken at 0.75 m above ground level to estimate light availability in each plot. In November 2000 (end of rainy season), I took one photograph in each quadrant of every plot using a Nikon Coolpix 950 digital camera and an 8 mm fisheye lens (Nikon, NY, USA). The images were analyzed using Gap Light Analyzer (Frazer et al. 1999) to obtain percent full sun light availability.

Soil cores (composites of five 10 cm-cores) were taken from each plot in early November 2000 and the samples analyzed for gravimetric water content and for available soil P using Mehlich 3 extractant (Mehlich 1984, Tran and Simard 1993), a strongly acidic extractant commonly used in soil testing laboratories to assess the amount of plant-available soil P (Pote et al. 1999). Because availability of soil nutrients, especially P, can vary temporally and even reverse ranks depending on season (Sollins 1998), I also sampled soils at the end of the dry season in June 2001. Soil P levels in the two seasons were correlated in the high ($r=0.91, p < 0.0001$) and intermediate ($r=0.81, p < 0.0001$) soil P sites. In the Carreta site, dry season soil P levels across all plots were uniformly close to the highest levels seen in the site during the wet season, likely because of reduced uptake by plants. For my analyses, I used soil P levels measured during the growing (i.e., wet) season.

To monitor growth and survival, seedlings were censussed and their diameter and height measured over approximately 28 months. Seedlings were censussed in August 2000, November 2000, June 2001, November 2002, May 2002 and November 2002. I used calipers to measure stem diameter ~3 cm above ground level, marking the spot with permanent ink to reduce subsequent measurement error.

On 30 August 2000, half of the plots were randomly selected in each site and fertilized by adding 96 mg P kg^{-1} soil in the form of rock phosphate. This fertilization level was based on the highest level of available P measured in the preliminary soil survey. However, subsequent soil sampling ~ 2 months later did not reveal any differences in soil P between the control and fertilized plots, likely due to the loss of P fertilizer following heavy rains shortly after fertilization. Further, there were no differences in growth rates or mortality between seedlings in control and fertilized plots for any species or site. Hence, data from fertilized and control plots were pooled for all analyses.

Data analysis

Seedling growth rates were calculated using a discrete time compound interest formula:

$$Diameter_t = Diameter_0 . [1 + G(res)]^t \qquad \text{Eq 1}$$

where $Diameter_t$ is seedling diameter (in mm) at time t (in years), $Diameter_0$ is the initial seedling diameter and $G(res)$ is growth rate as a function of resource availability.

Seedling growth was modeled using the Michaelis-Menten function, which has been commonly used to model growth as a function of light availability (Pacala et al. 1994, Kobe 1999):

$$G(res) = \frac{A * Light}{\left(\frac{A}{S} + Light\right)} \qquad \text{Eq 2}$$

where A corresponds with asymptotic growth and S corresponds with the slope of the growth function at low light.

Effects of soil P and soil moisture were tested by specifying A and S as functions of these resources which enables partitioning soil resource effects to low light or high light growth (Bigelow and Canham 2002, Kobe, in press):

$$G(res) = \frac{b * soil * Light}{\frac{b * soil}{S} + Light} \qquad \text{Eq 3}$$

$$G\ res = \frac{A * Light}{\frac{A}{c * soil} + Light} \qquad \text{Eq 4}$$

When both A and S are substituted to model a soil resource effect on both asymptotic and low light growth rates, the function reduces to a two parameter model of the form:

$$G(res) = \frac{soil * b * Light}{\left(\frac{b}{c} + Light\right)} \qquad \text{Eq 5}$$

Soil resource effects were also modeled using modified bivariate Michaelis-Menten functions of the form:

$$G(res) = \frac{A * Light * soil}{\left(\frac{A}{S_{light}} + Light\right) * \left(\frac{A}{S_{soil}} + Soil\right)} \qquad \text{Eq 6}$$

where $S_{resource}$ is the slope of the growth curve at the zero level of the subscripted resource. Thus, a set of seven equations was used to test functional relationships between resource availability and growth for each species. The basic equation incorporated only the effect of light on growth while the more complex equations added the effects of either soil P or soil moisture, as acting on growth at high light, low light, or both.

Equations were fit using the Gauss-Newton method in the non-linear procedure of Systat (SPSS Corp, Chicago, IL). Models were selected on the basis of Akaike's Information Criteria (Hilborn and Mangel 1997, Burnham and Anderson 2002). The AIC is an estimate of the expected, relative distance between the fitted model and the unknown mechanism that generated the data (Burnham and Anderson 2002). Unlike likelihood ratio tests (LRTs), AIC can be used for selection among non-nested models. The model with the lowest AIC is chosen as the best approximating model from the set of candidate models. In general, ΔAIC > 2 indicates poorer support for one model over the other (Burnham and Anderson 2002).

For Arboleda only, where maximum light availability was < 30% full sun and hence may have precluded asymptotic growth rates, I also modeled the effect of light on growth rate as a linear function:

$$G = M*light + constant \qquad \text{Eq 7}$$

Similarly, for the low soil P Carreta site, soil P was also modeled as affecting growth rate in a linear fashion. These linear models were not used in other sites because at higher availabilities, resources were not expected to have linear effects on species growth.

For *Astronium* and *Pachira,* because of very poor survival beyond the second dry season, I estimated growth models using data from the first 15 months only. Models for other species were based on growth over 28 months.

I used maximum likelihood and survival analysis to fit models of mortality as a function of resources. I used an exponential distribution of survival times and an exponential model for the hazard function, resulting in:

$$P(m) = 1 - e^{-\lambda * t} \qquad \text{Eq 8}$$

$$\lambda = A.e^{-(B*Light + C*soil\ P + D*soil\ moisture)} \qquad \text{Eq 9}$$

A, B, C and D are species-specific parameters estimated from the data (Kobe 1999). Models were fit using program code written in Borland Delphi by Richard Kobe. As before, I used AIC to select the best approximating model. I mapped uncertainty in parameter estimates (95% support) to uncertainty in functional relationships and assessed species differences as regions where 95% support for the functional relationships do not overlap.

To evaluate habitat-level species growth and mortality responses to variation in resource availability, I first tested if functional relationships were site-specific by comparing AIC values of models that did not differentiate between sites with models that were site-specific. Where AIC revealed site differences, I analyzed site-specific species responses to resource environments. For all species, to explore landscape-level species variation in growth and mortality, I fit models to data that were composited from all three sites.

Results

Growth

Light and soil resources influenced growth rates in all species (Table 4.3). All species showed strong positive growth responses to variation in light. *Astronium* and *Pachira* were more responsive to soil P, *Tabebuia* and *Cordia* to soil moisture, while *Dalbergia* responded to both soil P and soil moisture (Table 4.4).

In Carreta (the site with lowest P), three of five species growth rates responded to available soil P. Models incorporating effects of soil P on growth had lower AIC values (and hence were better fits) in *Astronium*, *Dalbergia* and *Pachira*. In these species, soil P acted to increase diameter growth rates at all light levels, although the form of the function was different in the three species. In *Astronium* and *Pachira*, soil P influenced both low-light and asymptotic growth rates (Eq 5) (Table 4.4) while in *Dalbergia*, a double Michaelis-Menten best characterized the relationship between soil P, light and growth rate. Hence, for all three of these species, light and soil P co-limited growth so that an increase in either light or soil P resulted in an increase in growth rate. In *Astronium*, Eq 3, characterizing multiplicative soil P effects only on high light growth also had substantial support. In *Tabebuia*, the unmodified Michaelis-Menten and the function modified to include soil moisture alone, or both light and soil moisture effects on growth provided equally good fits: ΔAIC for all three functions were < 1. In *Cordia*, light alone determined growth (Table 4.4).

Table 4.4 Maximum likelihood parameter estimates and 95% support intervals, samples size (N), coefficients of determination (r^2), and ΔAIC for all models with ΔAIC <2, relating diameter growth rates of species to resource availability. Because of poor survival beyond the second dry season, *Astronium* and *Pachira* models were estimated using data from only the first 15 months of the experiment.
AS – *Astronium*; CO – *Cordia*; DA – *Dalbergia*; PA – *Pachira*; TA – *Tabebuia*.
CA – Carreta (low P); OA – Ojo de Agua (intermediate P); AR – high P.

Sp.	Site	Model	A (95% support limits)	b (95% support limits)	S (95% support limits)	c (95% support limits)	S_l (95% support limits)	S_{soil} (95% support limits)	M (95% support limits)	I (95% support limits)	N	r^2	ΔAIC
AS	CA	b*P*light/ ((b/c) + light)		4.47 (1.14,5.33)		0.11 (-0.03, 0.25)					16	0.65	0
		b*P*light/ ((b*P/S) + light)		3.56 (1,79, 5.33)	0.31 (-0.10, 0.72)						16	0.68	1.85
	OA	A*light / ((A/c*P)+ light))	4.60 (2.20, 7.01)			0.12 (0.01, 0.23)					29	0.11	0
		b*P*light/ ((b*P/S) + light)		0.76 (0.32, 1.20)	0.66 (0.12, 1.12)						29	0.13	1.73
	AR	M*light + I							0.194 (0.07, 0.32)	-1.16 (-3.43,0.32)	10	0.62	--
CO	CA	A*light/ ((A/S) + light)	2.11 (1.92, 2.31)		0.11 (0.09, 0.13)						114	0.67	--
	OA	b*SM*light/ ((b/c) + light)		0.04 (0.03, 0.05)		0.02 (0.003,0.03)					95	0.41	0
		b*M*light/ ((b*M/S) + light)		0.04 (0.03, 0.05)	0.49 (0.10, 0.87)						95	0.41	0.38
		A*M*light/ ((A/S_l+ light)* (A/S_m+ M))	6.42 (-3.78, 16.62)				1.83 (-1.98, 5.6)	0.06 (0.02, 0.10)			95	0.42	0.72
		A*light / ((A/c*M)+ light))	1.84 (1.51, 2.19)			0.01 (0.01, 0.01)					95	0.41	1.04
	AR	A*light/ ((A/S) + light)	2.39 (0.03, 4.74)		0.08 (0.05, 0.11)						82		--

Table 4.4 (contd).

Sp.	Site	Model	*A* (95% support limits)	*b* (95% support limits)	*S* (95% support limits)	*c* (95% support limits)	S_l (95% support limits)	S_{soil} (95% support limits)	*M* (95% support limits)	*I* (95% support limits)	N	r^2	ΔAIC
DA	CA	A*P*light/ ((A/S_l+ light)*(A/S_p +P))	3.06 (2.27, 3.84)				0.17 (0.11, 0.22)	2.79 (1.52, 4.06)			125	0.54	--
	OA	b*M*light/ ((b/c) + light)		0.08 (0.06, 0.11)		0.01 (0.004,0.01)					64	0.53	0
		A*light/ ((A/c*M)) + light)	3.49 (2.54, 4.44)			0.01 (0.004,0.01)					64	0.52	1.42
	AR	M*light + I							0.10 (0.08, 0.13)	-0.63 (-1.04, -0.23)	58	0.51	--
PA	CA	b*P*light/ ((b/c)+ light)		1.13 (0.52, 1.73)		0.12 (-0.00, 0.24)					10	0.76	--
	OA	A*light/ ((A/c*P)+ light)	2.34 (1.08, 3.58)			0.16 (-0.17, 0.48)					20	0.05	0
		A*light/ ((A/S) + light)	2.38 (0.82, 3.94)		0.86 (1.30, 3.02)						22	0.03	0.41
	AR	M*light + I							0.19 (0.12, 0.26)	-0.96 (-2.24, 0.33)	19	0.64	--

Table 4.4 (contd).

Sp.	Site	Model	A (95% support limits)	b (95% support limits)	S (95% support limits)	c (95% support limits)	S_l (95% support limits)	S_{soil} (95% support limits)	M (95% support limits)	I (95% support limits)	N	r^2	ΔAIC
TA	CA	A*light/ ((A/S) + light)	2.66 (2.42, 2.90)		0.15 (0.12, 0.19)						130	0.59	0
		A*light/ ((A/c*M)+ light)	2.79 (2.54, 3.05)			0.01 (0.00, 0.01)					130	0.63	0.28
		A*M*light/ ((A/S_l+ light)* (A/S_m+ M))	3.24 (2.02, 4.47)				0.17 (0.11, 0.24)	0.73 (-0.78, 2.24)			130	0.63	0.88
	OA	b*M*light/ ((b/c)+ light)		0.15 (0.05, 0.25)		0.01 (0.00, 0.01)					81	0.60	0
		A*light/ ((A/c*M)+ light)	6.60 (2.25, 10.95)			0.01 (0.00, 0.01)					81	0.60	0.61
	AR	A*light/ ((A/S) + light)	11.04 (-35.18, 57.30)		0.09 (0.05, 0.13)						28	0.34	0
		M*light + I							0.08 (0.03, 0.12)	0.03 (-0.55, 0.61)	28	0.33	0.34

At very low levels of soil P, species' growth rates ranked as *Tabebuia* > *Cordia* > *Dalbergia* = *Pachira* (Figure 4.1A). Because of large overlapping confidence intervals on parameter estimates, growth rates in *Astronium* were not different from other species. With marginal increases in available soil P, growth rates of *Astronium* and *Pachira* increased substantially while *Dalbergia*'s growth rates increased to be equivalent with *Cordia*'s (Figure 4.1B). Growth rate ranks were thus maintained across all light levels but not across nutrient levels.

In Ojo de Agua (the site with intermediate soil P), in most species, growth rates depended on soil moisture rather than available soil P. In *Cordia*, *Dalbergia* and *Tabebuia*, Eq 5 characterizing linear soil moisture effects on both low- and high-light growth rates provided the lowest AIC, and hence the best fits (Table 4.3) although in all three, other equations modeling combined light and soil moisture effects also provided equal support. In *Astronium*, growth responded to both soil P and light, with soil P modifying growth rates at low light. Mean growth rates in this species were at least an order of magnitude larger than in other species but significantly different from only *Cordia*. A model with soil P modifying high light growth also had substantial support according to the AIC (Table 4.4). However, both models had poor fits, with $r^2 < 0.14$. In *Pachira* too, models incorporating combined light and soil P effects had the lowest AIC values but all models had poor fits, with r^2 values ~ 0.05.

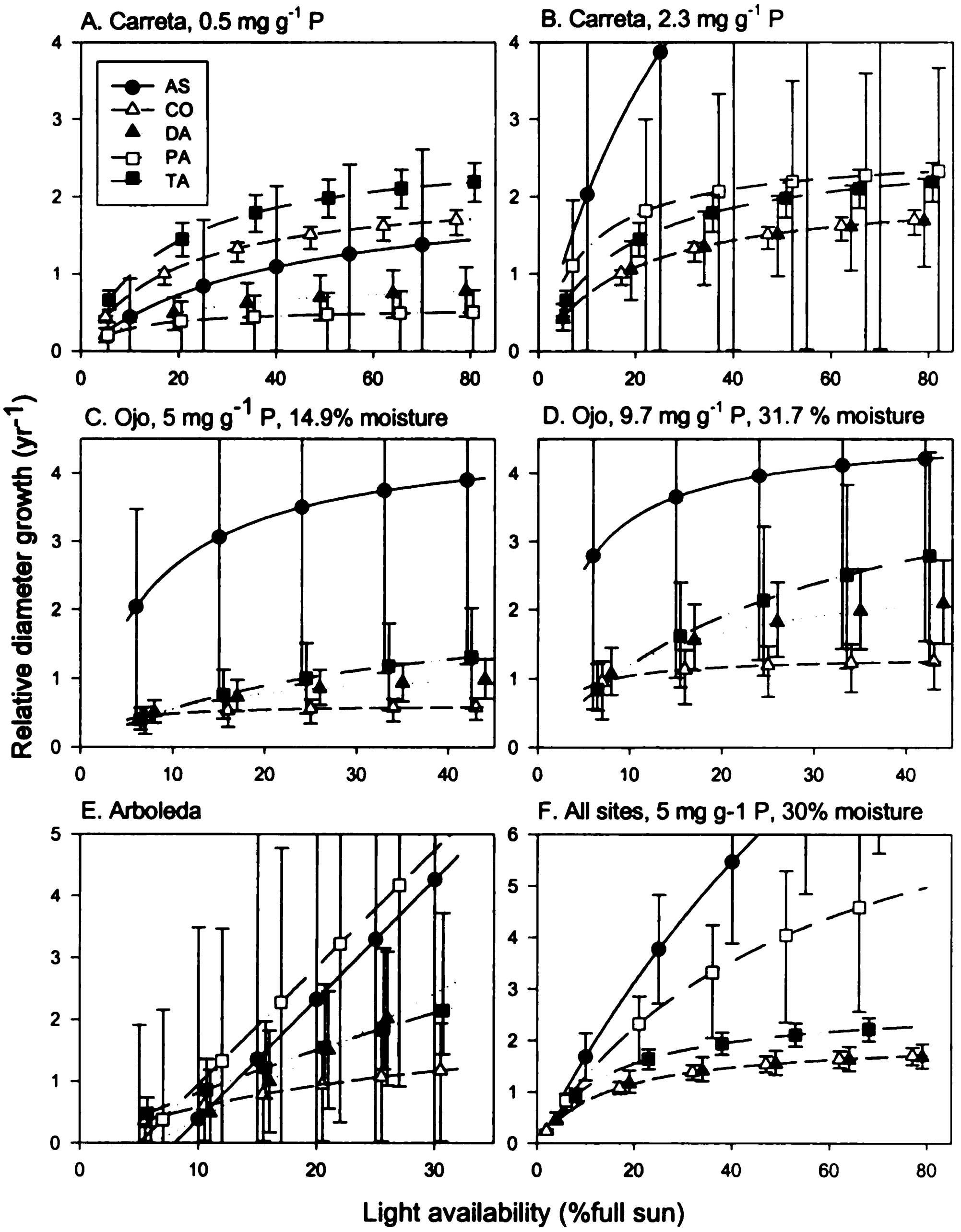

Figure 4.1 Models of diameter growth of seedlings (yr^{-1}) across light levels in the study sites: A) Carreta, with 0.5 mg kg^{-1} soil P; B) Carreta, with 2.3 mg kg^{-1} soil P
C) Ojo de Agua, with 5 mg kg^{-1} soil P and 14.9% soil moisture;
D) Ojo de Agua, with 9.7 mg kg^{-1} soil P and 31.7% soil moisture;
E) Arboleda; and F) across all sites, with 5 mg kg^{-1} soil P and 30% soil moisture.
Error bars represent 95% confidence limits at selected light levels.

Ranks of mean growth rate changed between low and high light in Ojo de Agua, although overlapping confidence intervals on estimated parameters indicate that these differences were not significantly different (Figures 4.1C and D). Asymptotic growth rates ranked as *Astronium* > *Tabebuia* > *Dalbergia* > *Cordia* although the difference was significant only between *Astronium* and *Cordia.*

In Arboleda, all species growth rates were determined by light availability alone (Table 4.4). *Cordia* and *Tabebuia* had a saturating relationship between light and growth rate (Table 4.4). The other species had a linear relationship between light and growth rate, likely because light levels in this site did not exceed 27%. Beyond ~7% light availability, *Pachira* and *Astronium* had the highest growth rates, and *Cordia* the lowest. At lower light levels, *Tabebuia* grew fastest (Figure 4.1E). However, none of these differences were significant (Figure 4.1E).

For all species, models that pooled data from all sites had substantially higher AIC values (and hence poorer fits) than site-specific models indicating that the relationship between growth and resources varied with site. However, we pooled data across sites to obtain growth-resource relationships that would approximate species behavior across broader resource variation than that provided by the site-specific models. Data pooled across sites revealed broad differences between the species which in most cases paralleled the site-specific responses. Pooling across sites, for both *Astronium* and *Pachira,* a Michaelis-Menten function modified to incorporate available soil P effects on asymptotic growth rate had the lowest AIC values (Table 4.5). *Dalbergia* and *Tabebuia* had growth rates dependent on both light and soil moisture while *Cordia*'s growth rate was governed by light alone (Table 4.5). At very low soil resource levels, such as at 0.5

mg kg^{-1} P and 15% soil moisture, and very low light availability, *Cordia* growth rates were higher than *Tabebuia* and *Pachira* growth rates while all other species growth rates were not significantly different from each other (figure not shown). When light availability was greater than ~10%, *Pachira* had significantly lower asymptotic growth rates than all other species. *Astronium* had nonsignificantly highest growth rates from 0% to 60% full sun after which *Tabebuia* growth rates were nonsignificantly higher. With marginal increases in soil resources, growth rates ranks changed so that under high light availability, *Astronium* = *Pachira* > *Tabebuia* > *Cordia* = *Dalbergia* (Figure 4.1F). Because soil moisture acted on low light growth in *Tabebuia* and *Dalbergia*, further increases in soil moisture led to these species achieving higher low light growth rates than *Cordia* and *Pachira*, although they were not significantly different from *Astronium* growth rates even at 80% soil water availability (figure not shown).

Table 4.5 Maximum likelihood parameter estimates and 95% support intervals, sample size (N), coefficients of determination (r^2), AIC and ΔAIC for all models with ΔAIC <2, relating relative diameter growth rates of species to resource availability across sites. Abbreviations are as in Table 4.4.

Sp.	Model	Param.	Parameter values (95% support limits)	N	r^2	ΔAIC
AS	*MM (HL soil P)*	*b*	4.404 (2.790,6.018)	57	0.25	--
		S	0.182 (0.135,0.230)			
CO	*MM*	*A*	2.040 (1.855, 2.224)	301	0.45	--
		S	0.135 (0.117, 0.153)			
DA	*MM (LL soil moisture)*	*A*	1.964 (1.732, 2.196)	250	0.27	0
		c	0.005 (0.004, 0.007)			
	Double MM (soil moisture)	*A*	3.243 (1.860, 4.625)	250	0.27	1.57
		S_l	0.253 (0.170, 0.337)			
		S_m	0.183 (0.077, 0.288)			
PA	*MM (HL soil P)*	*b*	1.680 (0.732, 2.628)	49	0.48	--
		S	0.153 (0.131, 0.174)			
TA	*MM (LL soil moisture)*	*A*	2.720 (2.481, 2.958)	234	0.53	--
		c	0.006 (0.005, 0.007)			

Mortality

In contrast to growth, where site-specific responses were important, mortality as a function of resource availability did not vary across sites: in all species, models that did not differentiate between sites had far lower values of AIC than site-specific models. In further contrast to growth responses which were sensitive to both light and soil resource availability, light alone affected mortality in all species (Figure 4.2). In all species, mortality decreased with an increase in light availability, except in *Pachira* where it increased slightly with light (Figures 4.2 and 4.3). Support bands (95%) on the relationship between mortality and light availability for *Astronium* overlap with those of *Pachira* indicating that *Astronium*'s mortality as a function of light was non-significantly lower than *Pachira* at all levels, although it was significantly higher than the remaining species. From 0-40% full sun, corresponding to understory to large gap light conditions, the mature forest species *Cordia* had significantly lower mortality than all other species. *Dalbergia* and *Tabebuia* had very similar mortality responses to light availability although from above 65% full sun, such as might be found in abandoned pastures, the slower-growing *Dalbergia* had lower mortality than the fast-growing *Tabebuia*.

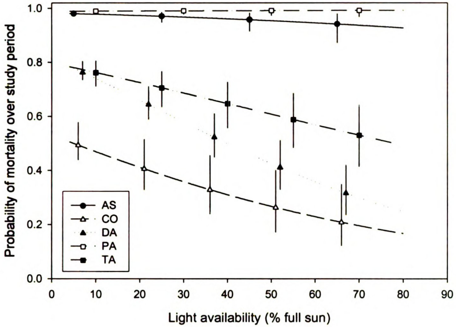

Figure 4.2 Mortality as a function of light availability for the five study species. Abbreviations are as in Table 4.4.

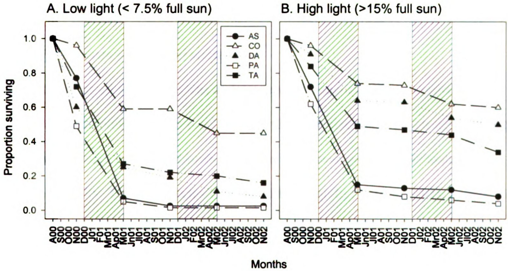

Figure 4.3 Time course of proportion of seedlings surviving under A. Low light (< 7.5% full sun) and B. High light availability (>15% full sun). Shaded portions show the dry season.

In all species, mortality was highest over the first dry season (Figure 4.3). Pooling data from all light levels and sites, across species, survival over the first wet season and the first dry season were strongly positively correlated (r=0.89, n=5, *p*=0.04) but there was no relationship between survival over the second wet season and the second dry season (r=0.43, n=5, *p*=0.46). These relationships held within higher light levels (>15% full sun). Under low light (< 7.5% full sun), there was also no relationship between first year wet and dry season survival among species (r=0.61, n=5, *p*=0.27). The lack of a correlation in low light is likely due to the change in ranks between survival at the end of the first wet season and at the end of the first dry season. In November 2000, before the onset of the dry season, species mortality decreased in the order *Cordia* < *Astronium* < *Tabebuia* < *Dalbergia* <*Pachira*. However, over the dry season, *Astronium* experienced 89% mortality, higher than any other species, so that it ranked only slightly above *Pachira* in survival in May 2001 (Figure 4.3).

Relationships between growth and mortality

Pooling data across all sites, there was an interspecific trade-off between growth under high resource availability and survival under low light availability. The species with the highest survival under low light, *Cordia*, also had the lowest growth rates under high resource availability. The two species with the fastest high light growth rates, *Astronium* and *Pachira*, had the lowest rates of survival under low light.

The strength of the trade-off varied with soil resource availability. In the low P Carreta, at very low soil P levels, the relationship was nonexistent (Figure 4.4A). As soil P increased marginally, a weak negative correlation developed between species growth under high light and survival under low light, the correlation increasing with soil P, but remaining nonsignificant (Figure 4.4A). On the other hand, in the intermediate fertility Ojo de Agua, the negative relationship between high light growth and low light survival was stronger than in Carreta, but still nonsignificant. As soil moisture increased, the relationship grew weaker (Figure 4B). In the high fertility Arboleda site, the trade-off was strongest (Figure 4.4C).

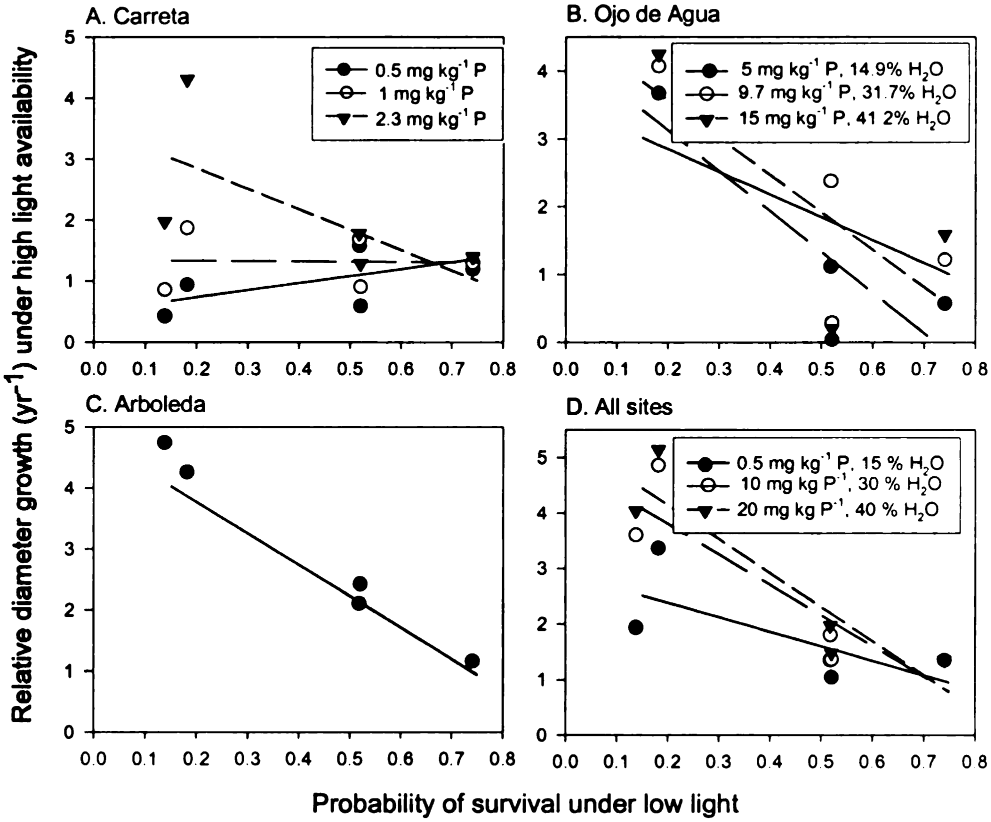

Figure 4.4 Trade-off between growth under high light availability (30% full sun) and survival under low light availability (5% full sun) as influenced by soil resources in: **A.** Carreta, with soil P varying (low: r = 0.57, p = 0.31; med: r = -0.03, p = 0.96; high: r = -0.68, p = 0.21); **B.** Ojo de Agua with soil moisture varying (low: r = -0.87, p = 0.12; med: r = -0.76, p = 0.24; high: r = -0.67, p = 0.33); **C.** Arboleda (r = -0.99, r = 0.0006); and **D.** Across all sites with soil P and moisture varying (low: r = -0.71, p = 0.17; med: r = -0.89, p = 0.04; high: r = -0.92, p = 0.03). Low, medium and high soil resource levels are represented by solid, long and short dashed lines respectively.

Across sites, species, and light levels, there was also a strong negative correlation between growth in the first wet season and survival in the first dry season ($r=-0.97$, $n=5$, $p= 0.0039$): *Astronium* and *Pachira* had the highest growth rates during the wet season and the lowest survival over the dry season. Conversely, *Cordia* had the lowest growth rates over the wet season but the highest survival over the dry season. However, contrary to Pearson et al. (2003), we found no relationships between wet season growth in high light and dry season survival in low light for either the first ($r=-0.51$, $n=5$, $p= 0.38$) or the second ($r=-0.33$, $n=3$, $p= 0.58$) wet and dry seasons.

I determined an index of performance of the five species based on their growth rates and survival in each site: the product of the probability of survival multiplied by growth rate of each species, representing ‘effective growth’, integrates survivorship and growth into a single metric and hence provides a better indicator of species performance than either taken singly. Figure 4.5 shows effective growth of the species at the three sites and reveals partitioning of both light and soil resources. In all cases, analyzing species performance in this manner revealed patterns different from those shown by growth alone, or mortality alone. In the low soil P Carreta site, at 0.5 mg kg^{-1} P, close to the lowest levels found in the site, *Cordia* dominates at light levels up to 60% and *Tabebuia* after that. Because of poor survival as well as relatively low growth, *Pachira* performed worst across all light levels in this site (Figure 4.5A). However, with marginal increases in soil P, performance ranks changed. *Cordia* retained first rank until 40% full sun, but *Astronium* was expected to perform better at higher light availability (Figure 4.5B). With further marginal increases in soil P at this site, *Astronium* dominated at all light levels. *Cordia* performance ranked second when light was < 40% but *Dalbergia* performed better at higher light levels (figure not shown). *Astronium* also dominated at all light levels in the intermediate fertility site Ojo de Agua when soil resource levels were close to or at the lowest levels found in the site (Figure 4.5C). However, performance ranks again changed with soil resources. When soil P was 9.7 mg kg^{-1} and moisture 31.7%, the average levels found in the site, *Cordia* was expected to outperform all other species when light was below ~15%, but *Dalbergia* dominated at higher light levels (Figure 4.5D). In the high fertility site Arboleda, *Cordia* and *Tabebuia* were expected to co-dominate at light levels below ~15%, but *Dalbergia* performed best at higher light levels (Figure 4.5E).

I also looked at species performance on a non-site specific basis (Figure 4.5F). At this scale too, performance ranks of the species depended on both light availability as well as soil resources. When soil P and moisture were very low, *Cordia* dominated and *Pachira* was expected to perform the worst at all light levels. However, with marginal increases in both soil resources, *Cordia* still performed better than other species below 35% full sun, but *Astronium* dominated at higher light levels.

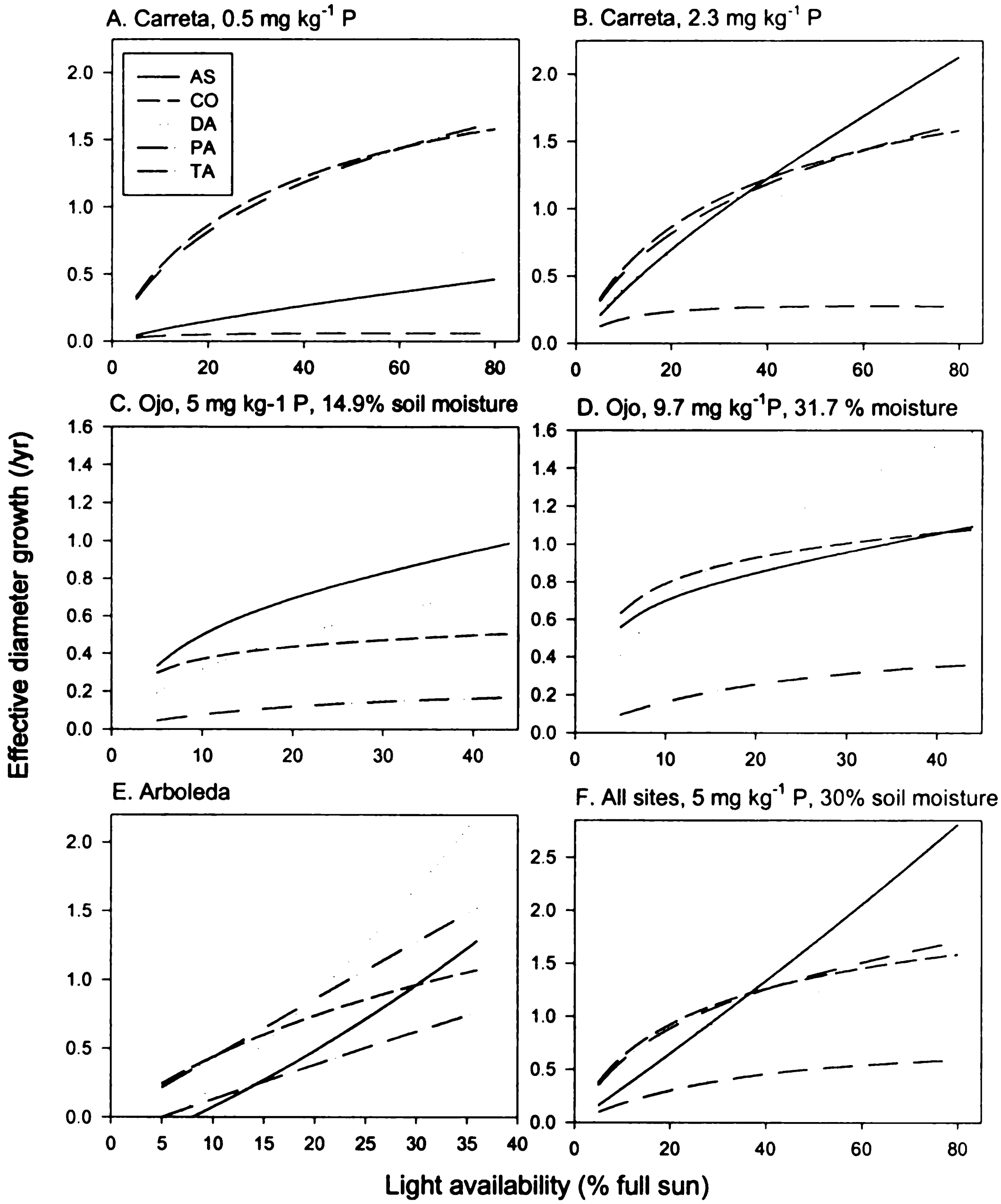

Figure 4.5 Effective growth (annual diameter growth of seedlings multiplied by survival), across light levels in
A) Carreta, with 0.5 mg kg^{-1} soil P; B) Carreta, with 2.3 mg kg^{-1} soil P
C) Ojo de Agua, with 5 mg kg^{-1} soil P and 14.9% soil moisture;
D) Ojo de Agua, with 9.7 mg kg^{-1} soil P and 31.7% soil moisture;
E) Arboleda; and F) across all sites, with 5 mg kg^{-1} soil P and 30% soil moisture.

Discussion

Resource effects on growth and mortality

Our results show clear effects of soil resources and light availability on the growth of seedlings. *Astronium* and *Pachira* consistently had higher growth rates under higher available soil P, *Dalbergia* and *Tabebuia* to soil moisture and *Cordia* largely to light availability.

In contrast to growth, mortality responded to light alone. Based on survival under low light, the mature forest species *Cordia* appears to be the most shade-tolerant of our species, while *Astronium* and *Pachira* are both shade-intolerants. The two most shade-intolerant species consistently had higher growth rates in sites with higher soil P. When both soil nutrient levels and light availability were high, *Astronium* and *Pachira* were also the fastest growing of our study species. The nutrient response by these shade intolerant, fast growers suggests *Astronium* and *Pachira* are competitors *sensu* Grime (1979), specializing in resource capture and growth. Higher nutrient uptake in these species would be required to support their higher growth rates (Chapin 1991), higher respiration (Bazzaz 1979) and shorter leaf life spans (Reich et al. 1992). However, *Astronium* is reported to be a slow grower (e.g., Piotto et al. 2004), and the abundance of long-lived *Astronium* saplings found in the understory (pers. obs.) suggest the species may be a 'late tolerator' (Oldeman, 1990, Grubb et al. 1996), requiring high light for establishment but able to persist for long periods in the shade as saplings and adults.

Based on species comparisons across sites, the most shade tolerant species, *Cordia*, can be characterized as a species that responds only to light, although in the intermediate fertility site, *Cordia* growth rates also increased in response to soil moisture.

Based on survival in low light, *Dalbergia* and *Tabebuia* appear to be intermediate in shade tolerance. *Tabebuia* consistently and *Dalbergia* largely respond to soil water rather than to nutrient availability. The lack of a growth response in these species, coupled with their lower growth rates under high light and soil resource availability, especially in *Cordia*, suggest these species may be stress tolerators, *sensu* Grime (1979). It has been hypothesized that shade tolerants take up P when its supply is greater than demanded by growth rates, using it when conditions allow higher growth rates (Burslem et al. 1995). Such 'luxury consumption' of P is an important mechanism by which species tolerate low P environments (Rorison 1968). However, in my species, growth rates in *Cordia*, *Dalbergia* and *Tabebuia* do not increase substantially under higher P even when light is not limiting, suggesting that this mechanism does not operate in these species.

All species except *Pachira* had greater survival under higher light availability. Increases in mortality with light, as seen in *Pachira*, are not uncommon in the tropics (e.g. Nunes et al. 1993, Gerhardt 1996a, Kobe 1999) where higher light can have deleterious consequences due to photoinhibition (Long et al. 1994). Alternatively, higher mortality under higher light availability could be due to an increase in herbivory in higher light environments. Although I fenced all plots to minimize vertebrate herbivory, we did not exclude invertebrate herbivory, which in this forest, is likely higher in higher light environments (Gerhardt 1996b). I did not directly measure leaf loss to herbivores, but field observations suggested young leaves in *Pachira* were more susceptible to herbivory than in other species.

Species trade-offs in growth rate and survival

I found a strong trade-off between species growth under high light availability and survival under low light availability. Such trade-offs in performance under different light environments have been found in both tropical (Kitajima 1994, Kobe 1999) and temperate trees (Kobe et al. 1995, Lin et al. 2002) and in temperate forests can facilitate species coexistence (Pacala et al. 1996). Furthermore, soil resources influenced the growth-survival trade-off. Because higher levels of available soil P led to higher asymptotic growth rates in fast-growing species only, while survival remained unaffected in all species, higher levels of available soil P led to stronger trade-offs between high light growth and low-light survival.

The trade-offs in growth and survival among the species led to clear differences in performance across resource environments (Kobe 1999). As light and soil resource availability change, the dominant species in each environment changes. Significantly, performance ranks of species in our study often did not change with light, but with soil resource availability. Further, our conclusions on species performance would have been very different if we had looked at growth alone, highlighting the importance of looking at mortality and growth simultaneously across gradients in resource availability. While previous studies have found significant differences among species in growth and survival in response to light and nutrient availability, differences in growth and mortality alone cannot be taken as evidence of resource partitioning. Differences in growth and survival can lead to coexistence only if changes in resource environments lead to changes in species' performance ranks (Latham 1992, Kobe 1999). Kobe (1999) showed light gradient partitioning among four neotropical seedlings. Here, I demonstrate the effects of soil resources as well as light on niche partitioning among tree seedlings in a dry tropical forest. The changes in performance ranks of species (taking both growth and survival into consideration) across spatial heterogeneity in resources provides the prerequisites for species coexistence through resource gradient partitioning in this forest.

Drought effects on growth rate and survival

As expected, I found great variation within species in growth and mortality across seasons. Mortality during the first dry season was greatest, between 25 and 80%, which is higher than that reported in a moist forest in Panama (Pearson et al. 2003) but similar to that reported in other dry forest studies (Gerhardt 1996). Seasonal drought had differential effects on mortality in the species. *Astronium* was more strongly affected by the first dry season, showing disproportionately high mortality compared to growing season mortality, an effect we would have missed in a short-term study.

Across all light environments, wet season growth and dry season mortality were strongly negatively correlated. However, unlike an earlier seasonal forest study (Pearson et al. 2003), there was no relationship between wet season growth under high light and dry season survival under low light because of disproportionately low *Astronium* survival over the first dry season.

Experimental limitations

First, because my fertilizer treatment to increase soil P did not result in differences in available soil P levels between control and fertilized plots, my study was correlative. My study focused on soil P as the nutrient that most likely limited growth in the study sites. However, levels of other soil nutrients also differed, though nonsignificantly, possibly leading to the differences in species growth among sites that could not be attributed to measured resources. Unmeasured variables such as rockiness could also have led to species growth differences among sites. Second, the expected high mortality during the dry season suggests dry season soil moisture is an important factor that influences survival in these species. However, due to logistical problems (unexpected changes in Costa Rican visa regulations), I was unable to measure soil moisture during the dry season. Third, although the Mehlich 3 extractant used is one of the most widely used extractants for measuring plant available P, chemical-extraction assays may not truly capture P availability to plants (Sollins 1998). Finally, although tropical dry forests are less speciose than rainforests, because my study used only five species, extrapolation to other species must be done cautiously.

Conclusions

This study demonstrates that seedlings growing in seasonally dry tropical forests experience multiple resource limitation, with their growth rates responding to availability of light, soil P or soil moisture. Light effects on survival combined with soil resource effects on growth led to a segregation of species along a resource axis so that no one species dominated all resource levels. The competitive hierarchy of species changed with both light and soil resource levels, and hence provided the prerequisite for coexistence of seedlings of these species through resource-based niches.

Acknowledgements

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Chapter 5

Stored carbohydrates in seedlings in a tropical dry forest: effects of light and soil resource availability

Introduction

The ability of some species to persist in low-resource environments usually trades off with their ability to grow fast under high resource availability (Kitajima 1994, Kobe et al. 1995, Schreeg et al. 2005). Thus, shade-tolerant species are able to survive low-light environments but are unable to grow as fast as shade-intolerants when light availability is high (Kobe et al. 1995, Lin et al. 2002). For species that maximize survival rather than growth, especially under low resource availability, allocation of resources to defense and to storage (Kitajima 1994, Kobe 1997) rather than growth could enhance chances of survival.

Allocation to storage of carbohydrates is generally at the expense of growth (Chapin et al. 1990). Stored carbohydrates in temperate seasonal forests are considered essential for species' survival and play important roles in respiration, overwinter survival and recovery from defoliation (Chapin et al. 1990, Kozlowski 1992). Storage is particularly important for survival through low-resource availability periods in seasonal environments (Chapin et al. 1990). In temperate forests, shade-tolerant species have higher total nonstructural carbohydrates (TNC) than shade-intolerant species (Kobe 1997), likely allowing them growth spurts in response to light gaps (DeLucia et al. 1998). TNC also enables recovery from herbivory (Chapin et al. 1990, Kozlowski 1992). It is hence likely to be important in tropical forests where herbivory is a leading cause of seedling mortality in low-light environments (Kitajima and Augspurger 1989). TNC has seldom been explored in tropical forests (Tissue and Wright 1995, Marquis et al. 1997) although recent studies have shown substantial variation in storage among species in seasonally dry tropical forests (Newell et al. 2002, Wurth et al. 2005).

In addition to species differences, spatial and temporal variation in resource availability can affect the storage of carbohydrates. Storage has been shown to increase with light availability (Mooney et al. 1995, Gansert and Sprick 1998) – although others have shown no light effects on storage (Kobe 1997, Canham et al. 1999) – and decrease with nutrient availability (Mooney et al. 1995). These studies suggest nutrient availability and light are both important in the field where trees can experience limitations in several resources that vary simultaneously.

In this study, I examined species differences in allocation to root TNC (generally the site of greatest TNC concentrations (Loescher et al. 1990)), and its relationship with growth and survival under varying light and soil resource availability among five co-occurring species in a seasonally dry tropical forest. The questions I addressed were:

1. What are the magnitudes of root TNC storage and how do they change with resource availability?
2. What effects does seasonality have on root TNC concentrations?
3. Is TNC positively related to survival and negatively related to growth?

Methods

Study site and species

The study area is in the Palo Verde National Park (10°21'N, 85°21'W), Guanacaste, Costa Rica, a tropical dry forest according to the Holdridge (1969) life-zone classification. The annual average temperature is 27.4°C and rainfall 1817 mm, with a pronounced dry season from December to May (Jimenez et al. 2001). The semi-deciduous forests in the 20,000 ha Palo Verde Park have approximately 65 tree, shrub and liana species per 1000 m^2 or 0.1 hectare (Gillespie 1999).

Table 5.1 Families, mean seed mass, leaf phenology and growth habits of the five study species.

Species	Family	Seed mass (mg)	Phenology [c]	Growth habit
Astronium graveolens	Anacardiacae	22.6 [a]	Leaf-exchanging; leaves emerge immediately after leaf shedding during the dry season.	Slow-growing, intermediate shade-tolerant
Cordia gerascanthus	Boraginaceae	44.9 [b]	?	Intermediate shade-tolerance, late successional
Dalbergia retusa	Fabaceae	77.4 [a]	Brevideciduous; photoperiod increase induces leaf flush end March	Slow-growing, intermediate shade-tolerant, late successional
Pachira quinata	Bombacaceae	22.4 [a]	Deciduous; photoperiod increase induces leaf flush end Apr/early May	Fast-growing, shade intolerant, early successional
Tabebuia rosea	Bignoniaceae	22.6 [a]	Deciduous; leaf flush after rainfall	Fast-growing, shade-intolerant, early successional

Sources: *a*: this study; *b*: Opler et al. 1975; *c*: Borchert et al. 2002.

I selected five species for the study – *Astronium graveolens* Jacq., *Pachira quinata* (Jacq.) WS Alverson, *Cordia gerascanthus* L., *Dalbergia retusa* Hemsl. and *Tabebuia rosea* (Bertol.) DC. – based on seed availability and to present a range of seed sizes and growth habits (Table 5.1). Species appear to show some soil resource affinities (Table 4.1, Chapter 4) though all co-occur as adults in the secondary forests of the Park. *Astronium* is a leaf-exchanging species, *Dalbergia* is brevideciduous, (Rivera et al. 2002) and all other species are deciduous as adults although the period of leaflessness varies (Table 5.1). Hereafter, species are referred to by their genus names only.

Experimental methods

I used three sites varying in soil phosphorus (P) availability for my study. All sites were in secondary forest areas and within 3-6 km of each other. The sites provided a natural gradient in soil P with site Carreta having the lowest, Ojo de Agua intermediate and Arboleda the highest levels of soil P. As part of an experiment investigating resource effects on growth and survival in these species (Iyer et al., in prep., Chapter 4), I established 140, 70 and 156 experimental plots, respectively, in each site, stratified across light and soil P availability. Each plot measured 1.5 x 1.5m, with a distance of at least 1 m between plots. To maintain a consistent light and soil resource environment, vegetation was cleared from the plots and resprouts cut back at intervals. To minimize vertebrate herbivory, all plots were fenced to a height of 1.25m.

In June-July 2000, I germinated seed of *Dalbergia, Tabebuia* and *Pachira.* We collected *Dalbergia and Tabebuia* seed from the vicinity and purchased *Pachira* seed from a commercial source that collected seed locally. To promote mycorrhizal inoculation, I germinated seeds in flats containing a mixture of 9:1 sand:field soil, with each site contributing 1/3 of the field soil component. Germinants were transferred to plastic nursery bags with soil from the site where they would eventually be transplanted. I collected new germinants (i.e., seedlings retaining cotyledons) of *Astronium* and *Cordia* from the field and transplanted them into bags using the same procedure as for the germinated seed. Seedlings of all species were transplanted into the experimental plots over two weeks starting 1 August 2000. Each plot had 2-4 individuals of each species, and hence 10-12 individuals planted in randomly chosen positions of a 4x4 grid with positions separated by 50 cm, with some grid positions remaining vacant.

To test for relationships between allocation and resource availability, I measured light, soil P and soil moisture in each transplant plot as described in Chapter 4. Resource levels in each site are as in Tables 4.2 and 4.3, Chapter 4.

On 30 August 2000, half the plots (randomly selected) in each site were fertilized by adding 96 mg P/kg soil in the form of rock phosphate. This fertilization level was based on the highest level of available P measured in the sites during a preliminary soil survey. However, subsequent soil sampling 2.5 months later did not reveal any differences in soil P between the control and fertilized plots. Further, there were also no differences in growth rates, mortality or storage of carbohydrates between seedlings in control and fertilized plots for any species or site. Hence, data from fertilizer and control plots were pooled for all analyses.

To investigate intra- and inter-specific variation in root storage, and to determine how allocation responds to seasonality, I harvested seedlings at the end of the first three wet seasons that the seedlings experienced: November 2000, November 2001 and November 2002. We had one harvest at the end of their second dry season in mid-May 2002, a week before the first rainfall of the wet season. Because of the lower number of *Astronium* and *Cordia* seedlings available for transplanting at the start of the experiment, we harvested seedlings of *Dalbergia*, *Pachira* and *Tabebuia* only. The first three harvests were restricted to the high and low P sites only. Large-scale mortality in *Pachira* led to very small sample sizes from the second and subsequent harvests. To allow broader species comparisons, I also harvested *Cordia* seedlings in May 2002. For the final harvest in November 2002, I harvested seedlings of all five species from all three sites.

Seedlings to be harvested were randomly chosen. Although I attempted to excavate all seedlings' root systems completely, because of the difficulty involved in excavating roots from the high-clay soils, we only excavated roots to a depth of about 80cm. Seedlings were washed in tap water, separated into leaves, stems and roots and dried in a plant dryer at ~ 75°C for two days. Dried roots were transported to Michigan State University where they were pulverized with a ball mill (Kinetic Laboratory Equipment Co., California) before TNC extraction and analysis.

Laboratory analyses

I analyzed TNC in roots of harvested seedlings as described in Chapter 3. Briefly, I used hot alcohol to extract soluble sugars which were then analyzed with a phenol-sulfuric acid assay (Dubois et al. 1956). Extraction residues were enzymatically digested and analyzed with a glucose-specific colorimetric assay (Roper et al. 1998). TNC concentration was calculated as the sum of glucose equivalents of the soluble sugars and starch measured in each sample.

Data analysis

I developed a set of candidate models to characterize root TNC concentrations for each species, harvest and site combination as functions of light. I used a linear model with a constant to characterize a constant fraction of whole plant mass being allocated to root storage or structure.

$$TNC\ concentration = a{*}Resource + b \qquad \text{Eq. 1}$$

where *Resource* is either light availability, soil P or soil moisture. I also modeled storage as a power function:

$$TNC\ concentration = c*(Resource^{\wedge}d) \qquad \text{Eq. 2}$$

Eq. 2 is the allometric scaling model used by Niklas and Enquist (2001), where $d > 1$ implies allocation to root TNC (or structure) increases disproportionately with whole plant mass while $d < 1$ less than proportionate increases with plant size. Soil P and soil moisture were modeled as modifying light-driven allocation by substituting for the parameters a and b, or c and d in Equations 1 and 2, respectively, with functions of the form $(f*soil)$ where *soil* represents either soil P or soil moisture.

Model parameters were estimated using maximum likelihood methods (Hilborn and Mangel 1997). I assumed a normal error distribution, testing this assumption with probability plots and G-tests. Models were selected on the basis of Akaike's Information Criteria (Hilborn and Mangel 1997, Burnham and Anderson 2002), with a correction for sample size (AIC_c, Hurvich and Tsai 1989, Burnham and Anderson 2002). The model with the lowest AIC_c is chosen as the best approximating model from the set of candidate models for each relationship. In general, a difference of more than 2 units in the AIC_c of two models indicates poorer support for one model over the other (Burnham and Anderson 2002).

I tested if harvest time affected the relationship between allocation to TNC and resources differently by using dummy variables. For example, to test if harvests two and four differed, for a linear relationship between root TNC and resources, I used equations of the form:

$$Root\ TNC\ conc = [A_2*(Resource) + B_2]*v_2 + [A_4*(Resource) + B_4]*v_4,$$

where A_2, B_2 and A_4, B_4 are the estimated parameters for harvests 2 and 4, respectively, and v_2 and v_4 are dummy variables that take on values of 0 or 1. I tested functional differences between harvests by evaluating ΔAIC_c between harvest-specific and combined equations.

I did not model relationships in cases where sample sizes were less than 9. Equations were fit using the Gauss-Newton method in the non-linear procedure of Systat (SPSS Corp, Chicago, IL).

Results

Across species, two-year old seedlings had root TNC concentrations ranging from 10% to 39% (Table 5.2). In all sites, the fast-growing shade-intolerant species *Pachira* had the highest root TNC concentration while the mature forest species *Cordia* had the lowest concentrations. Starch was the dominant form of stored carbohydrate in all species except *Tabebuia*, which had starch concentrations close to zero in earlier harvests with very slight increases in later harvests (Figure 5.1). The effects of whole plant mass on root TNC concentration were not very prevalent. In most species-harvest combinations there was no correlation between the two metrics (data not shown). Root TNC concentration and whole plant mass were correlated only in the following cases: *Dalbergia* in the first harvest in the low P site (n=15, r=0.73, p=0.0019), and in the first two harvests in the high P site (n=13, r=0.78, p=0.0015; and n=12, r=0.84, p=0.0007, respectively); *Tabebuia* in the second harvest in the low P site (n=11, r=0.77, p=0.0052) and in the final harvest in the high P site (n=9, r=0.81, p=0.0084).

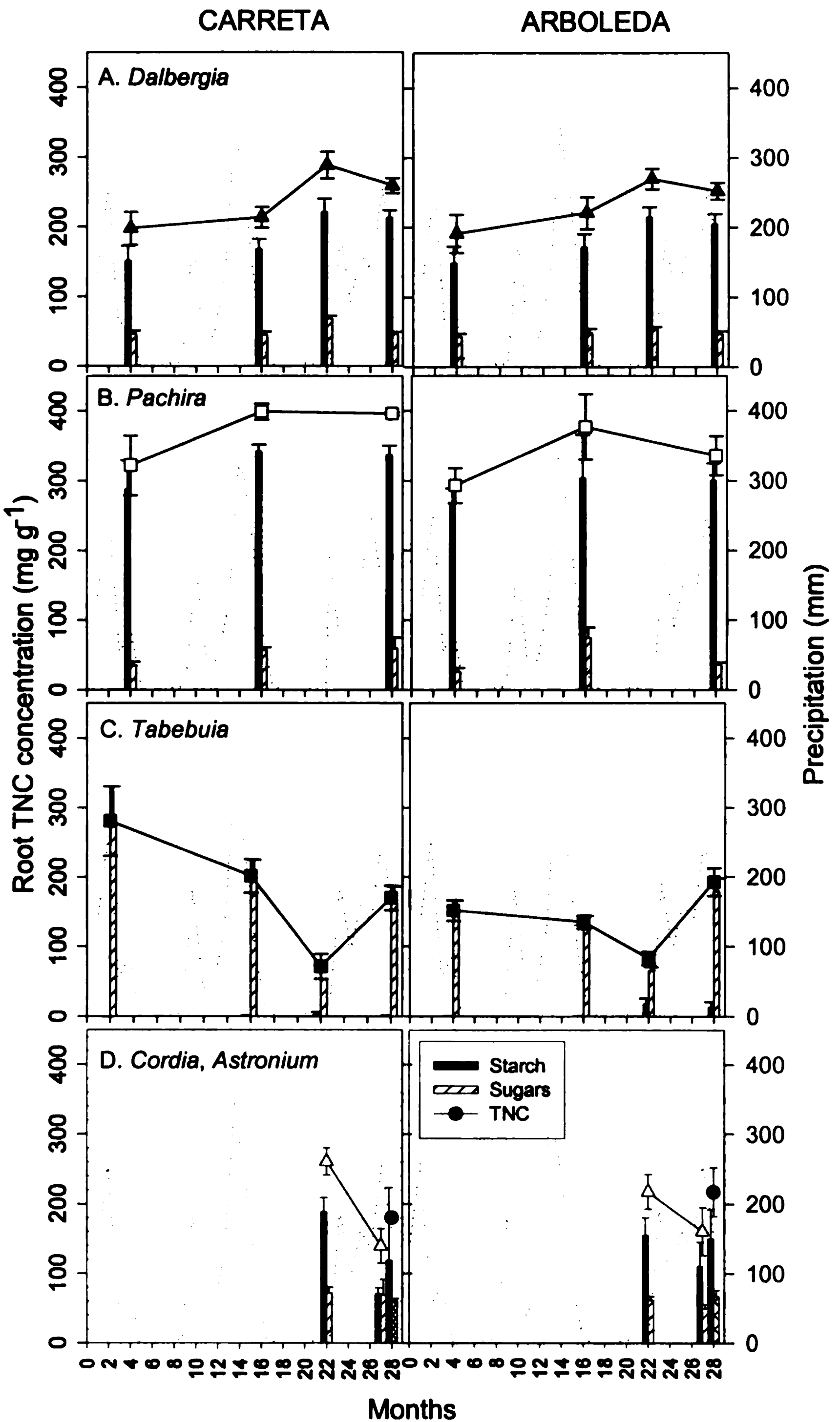

Figure 5.1 Mean (+ SE) concentrations (mg g $^{-1}$) of starch, simple sugars, and TNC in roots of the study species A. *Dalbergia,* B. *Pachira,* C. *Tabebuia* and D. *Cordia* and *Astronium* (the latter represented by a filled circle at a single point at the 28th month only) at each harvest in low P Carreta (left column) and high P Arboleda (right column). Dotted lines represent monthly precipitation (mm).

Table 5.2 Mean ± SE of root carbohydrate concentrations (mg g^{-1} TNC) at the end of the third wet season (November 2002). Sample sizes are given in parentheses. Letters denote significant differences between species in each site (Tukey's HSD, $p<0.05$).

Site	*Astronium*	*Cordia*	*Dalbergia*	*Pachira*	*Tabebuia*
Carreta	179.98^{bc} (2)	139.5±24.5^{c} (15)	259.0±10.7^{ab} (15)	396.0^{a} (2)	169.9±17.5^{c} (15)
Ojo de Agua	196.9±14.9^{ab} (8)	101.1±17.8^{b} (11)	271.3±19.6^{a} (10)	275.2±84.7^{a} (4)	224.6±21.8^{a} (10)
Arboleda	216.9±35.0^{abc} (5)	160.3±34.3^{c} (12)	252.3±12.1^{ab} (13)	335.6±28.0^{a} (5)	192.7±20.2^{bc} (9)

Resource effects across harvests

Across species, sites, and harvests, resource availability explained between 16 and 85% of variation in root TNC concentrations (Appendix A.5, Figures 5.2, 5.3 and 5.4). Light explained between 40 and 50% of the variation in root TNC concentrations at the end of the first wet season in most species-site combinations (data not shown). At the end of the second wet season, soil resources acted together with light in influencing root carbohydrate storage (Appendix A, Figures 5.2 and 5.3). However, by the end of the third wet season (fourth harvest), light had an effect on root TNC concentrations only in *Tabebuia*. In the high soil P site, light interacted with soil P to increase root carbon storage in *Tabebuia* (Figure 5.3) while in low P Carreta, light similarly influenced root carbohydrate storage in the second and fourth harvests at the end of the second and third growing seasons respectively: AIC_c for the relationship was lower when data from the two harvests were pooled than when the two harvests were analyzed separately (Appendix, Figure 5.3). In *Cordia*, soil P had a very weak negative effect on root TNC concentrations in high P Arboleda, while soil moisture had a positive effect on root TNC concentrations in low P Carreta (Figure 5.4). In *Dalbergia*, resource availability did not have any effect on root TNC concentration in Arboleda, but soil moisture had a positive effect on root TNC in Carreta (Figure 5.2).

Dry season root TNC concentrations were only weakly correlated with resource availability (Appendix A.5, Figures 5.2, 5.3 and 5.4). In *Dalbergia*, soil moisture had a weak negative effect on root TNC concentrations at the end of the dry season (third harvest) in Arboleda while in Carreta, resource availability did not influence root TNC (Figure 5.2). In both *Cordia* and *Tabebuia*, variation in resources did not have any effect on root TNC in the high soil P Arboleda. In low P Carreta, increased soil P led to weak increases in root TNC in *Cordia* while in *Tabebuia*, soil P and light interacted to produce a weak positive effect on root TNC (Figures 5.3 and 5.4).

Of the three species for which we measured root TNC concentrations at the end of the dry season as well as at the end of the wet season, *Cordia* and *Dalbergia* had higher TNC levels and *Tabebuia* lower TNC concentrations at the end of the dry season than at the end of the rainy season (Figure 5.1); *Dalbergia* had highest and *Tabebuia* the lowest root TNC concentrations, respectively, in May in both sites.

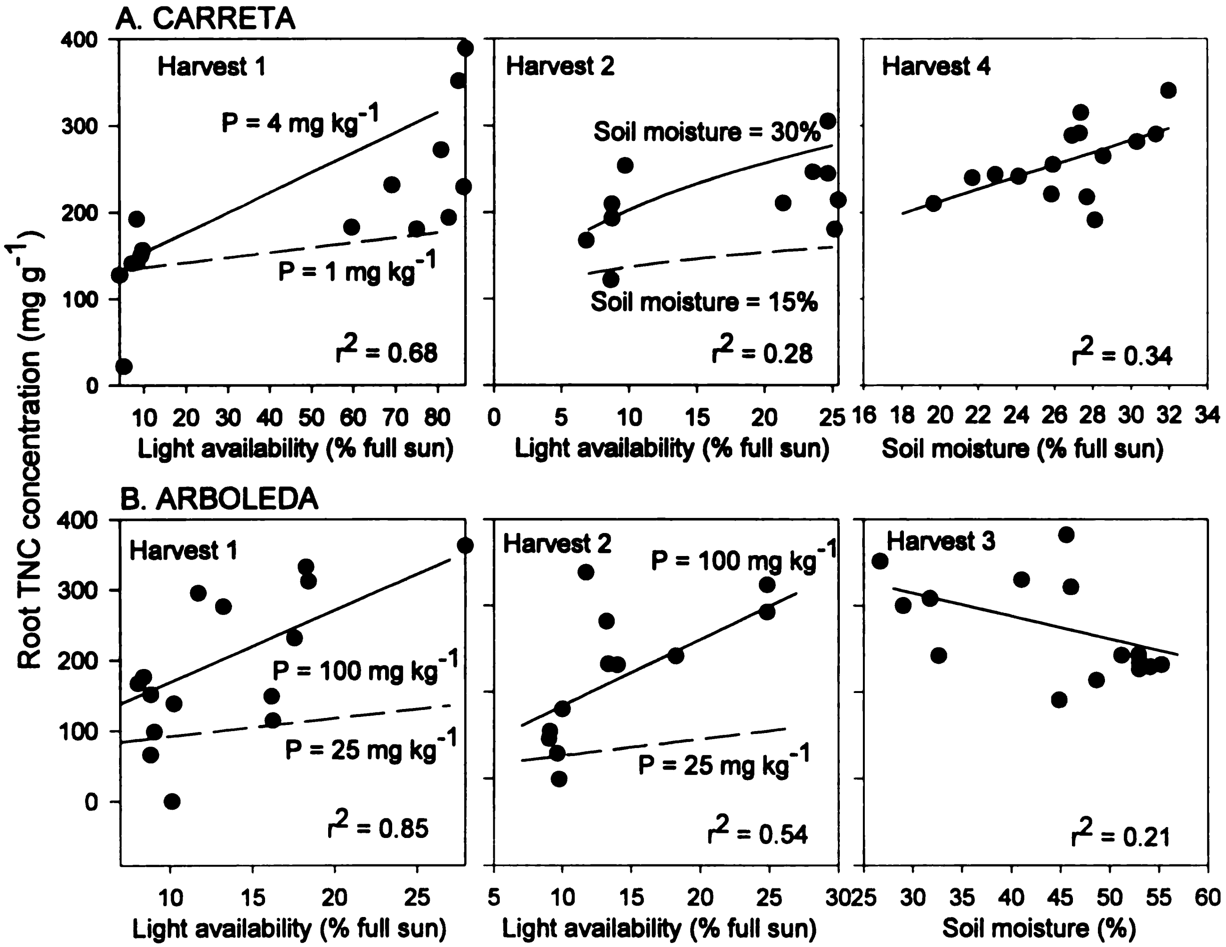

Figure 5.2 Relationship between root TNC concentration (mg g^{-1}) and resource availability in four harvests of *Dalbergia* in Carreta (low soil P) and Arboleda (high soil P). Lines represent best-fit equations.

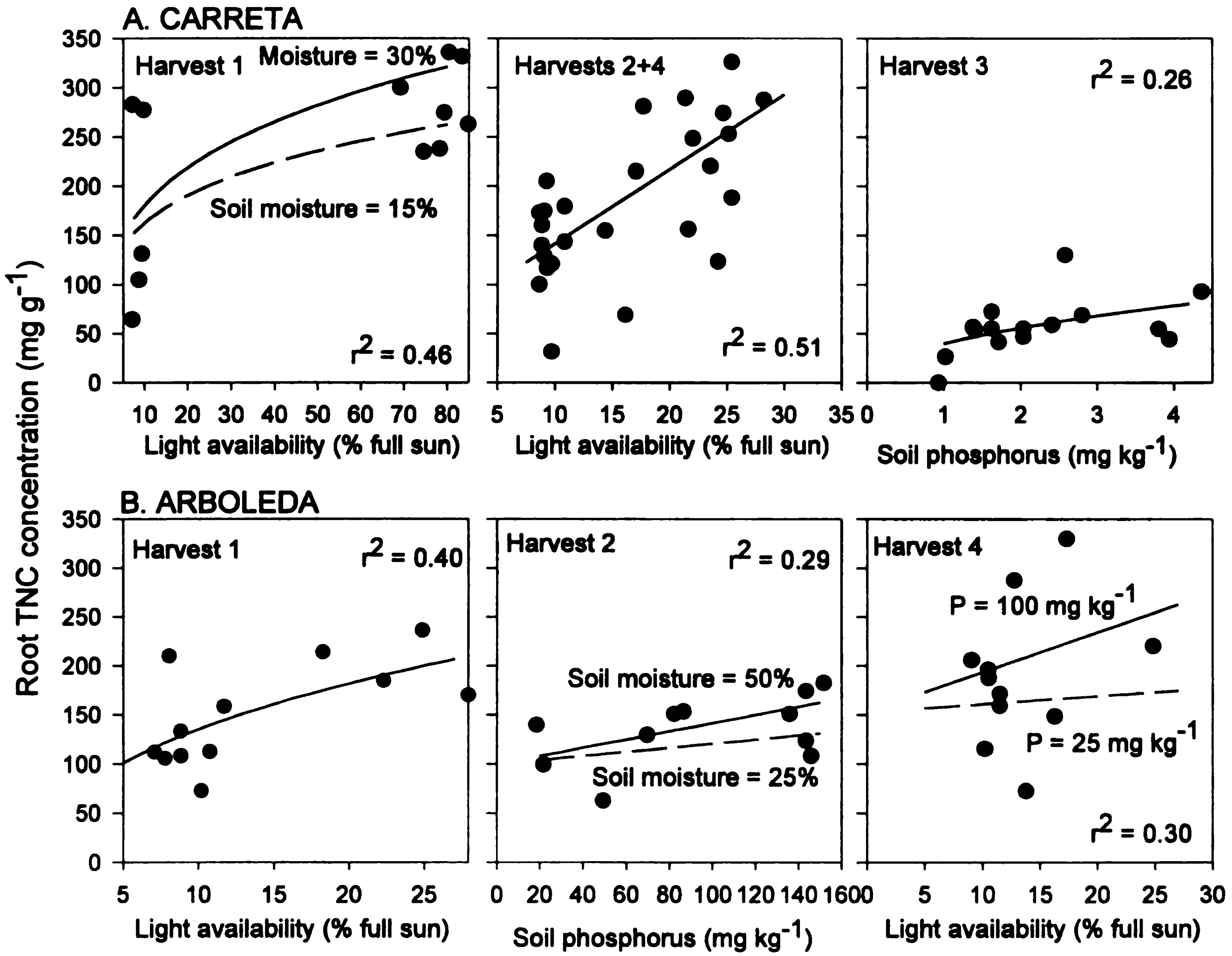

Figure 5.3 Relationship between root TNC concentration (mg g^{-1}) and resource availability in four harvests of *Tabebuia* in Carreta (low soil P) and Arboleda (high soil P). Lines represent best-fit equations.

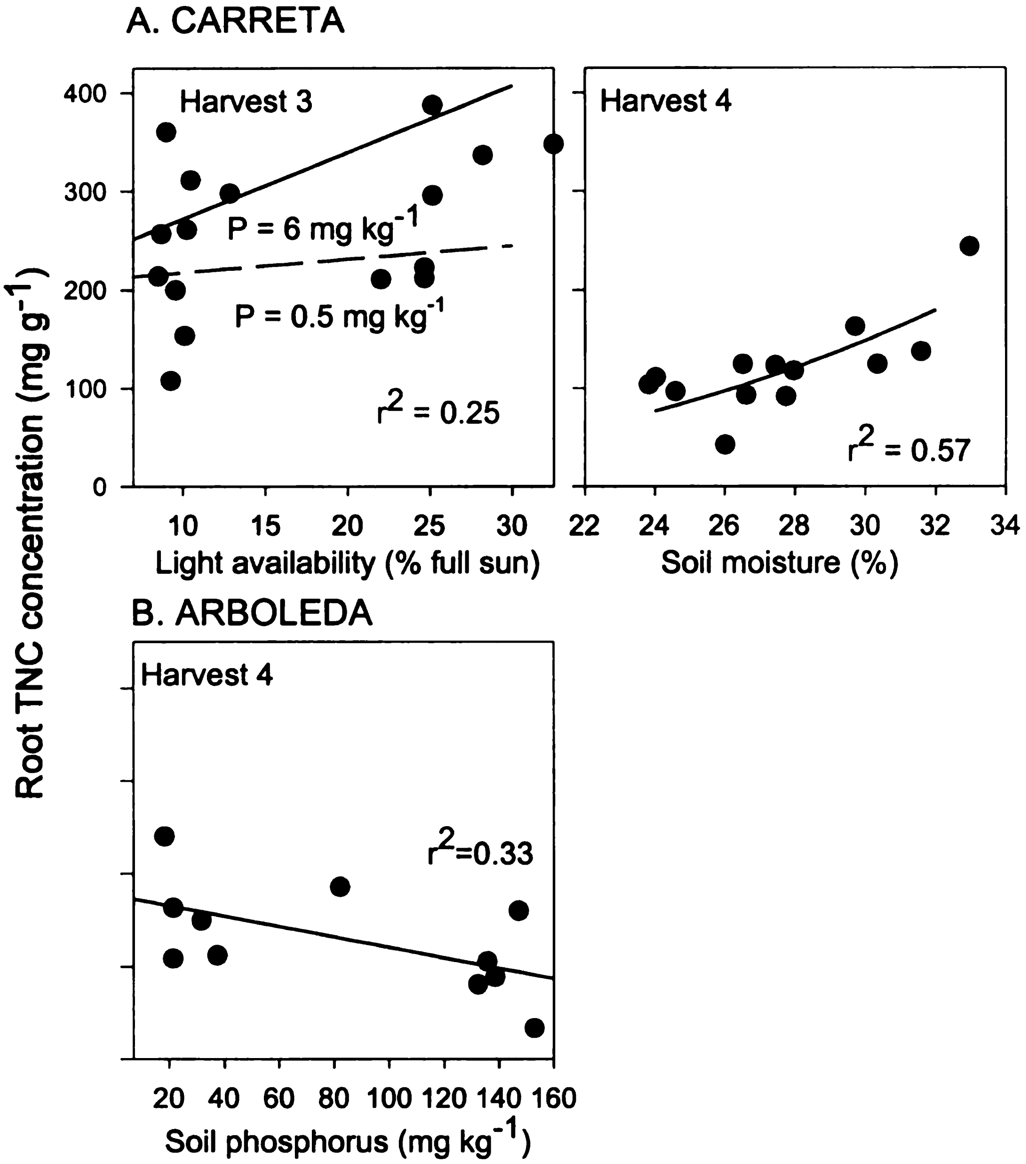

Figure 5.4 Relationship between root TNC concentration (mg g^{-1}) and resource availability in two harvests of *Cordia* in Carreta (low soil P) and Arboleda (high soil P). Lines represent best-fit equations.

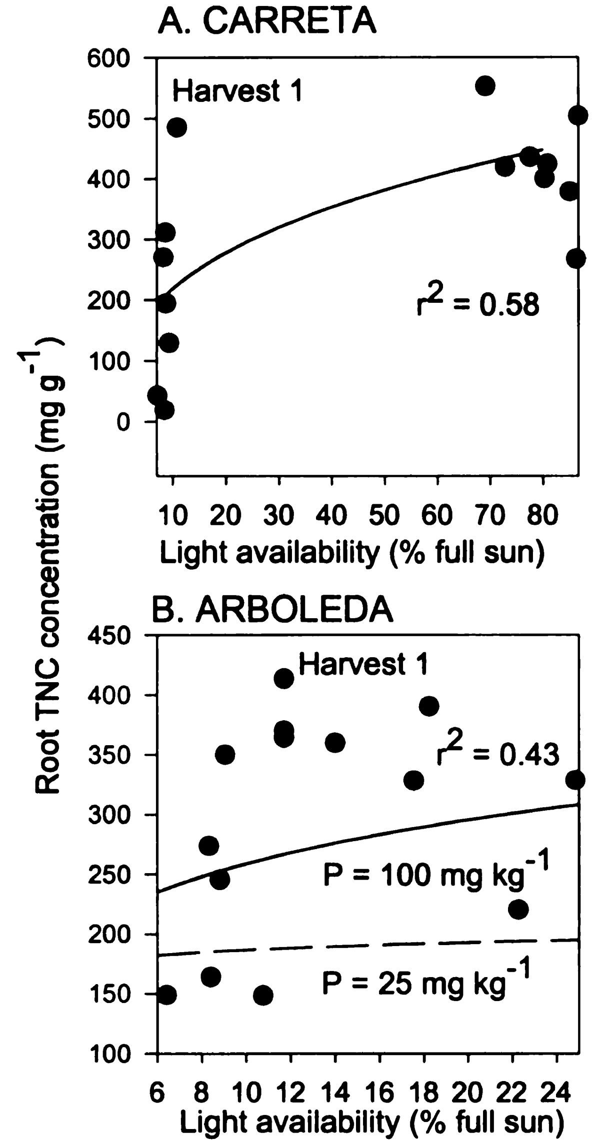

Figure 5.5. Relationship between root TNC concentration (mg g^{-1}) and resource availability in the first harvest of *Pachira* in Carreta (low soil P) and Arboleda (high soil P). Lines represent best-fit equations.

TNC, growth and survival

Species' mean root TNC concentrations at the end of the experiment and species' mean diameter growth rates over the experiment were negatively correlated in the low P site Carreta, positively correlated in the high P site Arboleda, and were not correlated in the intermediate soil P site Ojo de Agua (Figure 5.6). The lack of a consistently significant correlations could arise from the small number of species in our study. However, under low light (<10% full sun), there is no relationship between growth and TNC in any site, while under high light (>15% full sun), species' mean root TNC concentrations and species' mean diameter growth rates are positively correlated only in the high soil P site ($n = 5, p = 0.87, p = 0.05$).

Contrary to expectations, species' mean root TNC concentrations at the final harvest and species' survival over the length of the experiment were not positively correlated: there were weak nonsignificant, negative correlations between the two variables in all three sites (Figure 5.7). Analyzing these relationships under low and high light availability also consistently showed weak, negative correlations between mean root carbohydrate stores in species in November 2002 and survival until that time (data not shown).

Species' mean root TNC concentrations at the end of the second wet season (November 2001) and survival through the following dry season were negatively correlated in low P Carreta ($n=3, r=-0.99, p=0.07$) and also with survival through the next wet season ($n=3, r=-0.99, p=0.09$). In the high P Arboleda, there was a weak trend towards a positive correlation between wet season TNC and survival through the following year (n=3, r=0.87, *p*=0.33).

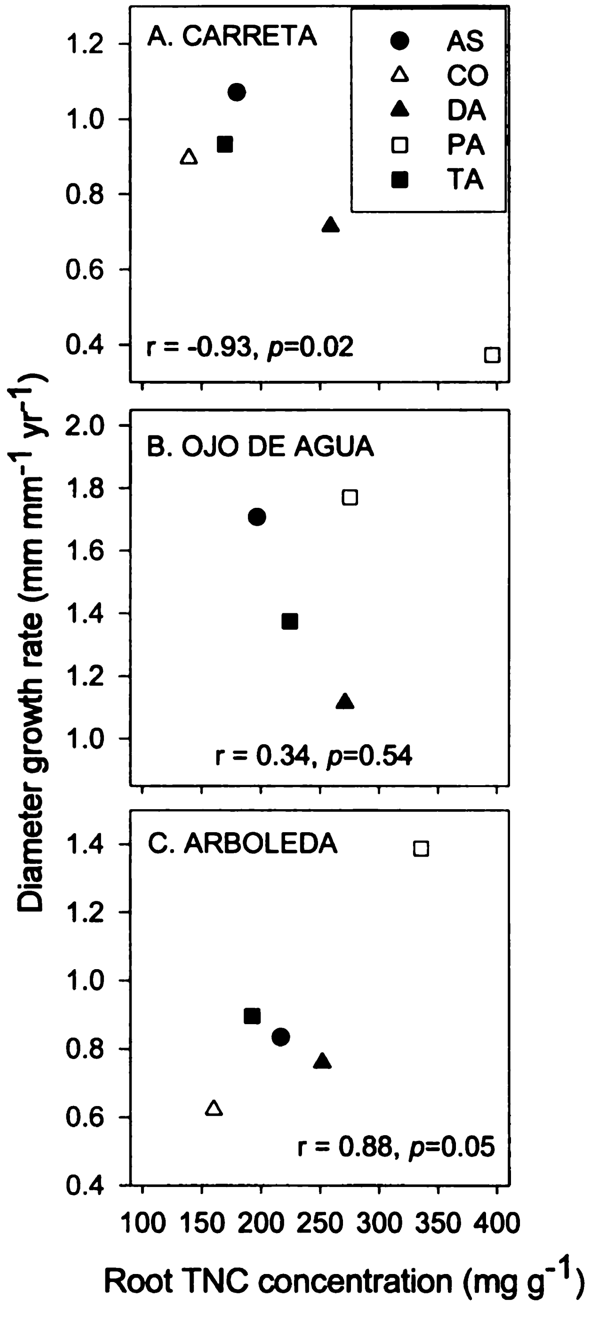

Figure 5.6 Relationship between species' mean diameter growth rates (mm mm^{-1} yr^{-1}) and mean root TNC concentrations (mg g^{-1}) at the end of the third wet season in A. Carreta (low soil P); B. Ojo de Agua (intermediate soil P); and C. Arboleda (high soil P).

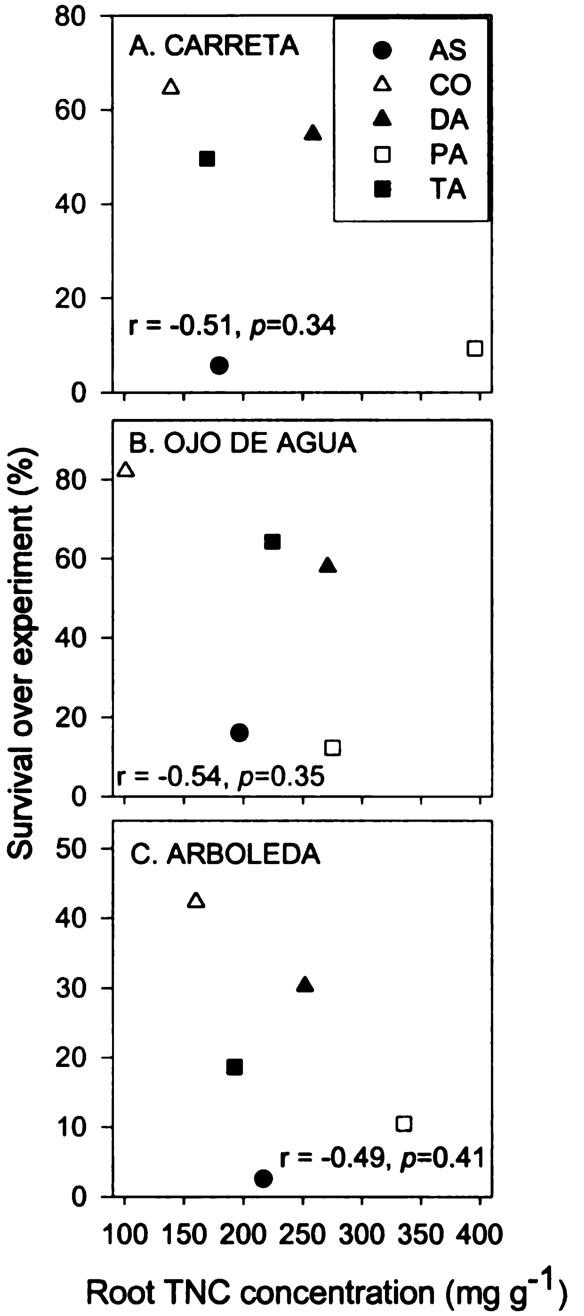

Figure 5.7 Relationship between species' survival (%) and mean root TNC concentrations (mg g^{-1}) at the end of the third wet season in A. Low soil P Carreta, B. Intermediate soil P Ojo de Agua, and C. High soil P Arboleda.

Discussion

All species had substantial proportions of stored carbohydrates in their roots, ranging from ~10 to 40% at the final harvest. These levels are comparable with or higher than those reported for some moist tropical forest tree species (Newell et al. 2002) and rainforest shrubs (Marquis et al. 1997) and are generally higher than those found in other studies in moist (6-8% in Wurth et al. 2005) and seasonal forests (4-6% in roots of plantation species; Latt et al. 2001). Some of these differences may be because Wurth et al. (2005) sampled only 10-15 mm diameter roots, likely leading to their result of lower TNC concentrations. Temperate forest seedlings also have comparable levels of root TNC concentrations (e.g., Canham et al. 1999, Chapter 2).

Starch was the predominant form of stored carbohydrate in four of the five species, as is the case in most species (Kozlowski 1992). However, *Tabebuia* roots had very low levels of starch. The negligible levels of starch in this species are likely due to water deficits which cause decreased starch and often, an increase in soluble sugars (Kramer 1983), which maintain osmotic balance and turgor under water stress (Ritchie 1982).

Temporal changes

There were contrasting temporal patterns in TNC concentration among species. Contrary to expectations, mean root TNC concentrations in *Dalbergia* increased substantially over the dry season (from November 2001 through May 2002). Although I do not have data to directly examine dry season changes in *Cordia* root TNC concentration, for both *Cordia* and *Dalbergia,* TNC root concentrations were higher at the end of the dry season (May

2002) than at the end of the wet growing season (November 2002), suggesting that TNC pools are depleted during what is considered to be the growing season and build up during the dry season. It is possible that because of infrequent sampling, I missed further TNC concentration increases at the end of the rainy season in November 2001 and 2002. However, the magnitude of the increase in TNC suggests this is unlikely. Increases in TNC during seasonal droughts have been reported in other seasonal tropical forest species (Newell et al. 2002, Latt et al. 2001, Wurth et al. 2005). It is thus more likely that the increase in TNC is brought about by a cessation of growth but not photosynthesis in these species (Kitajima et al. 1997, Wurth et al. 2005). This is consistent with the link between leaf phenology and dry season root TNC in my study species. Of the two species for which dry season root TNC was higher, *Dalbergia*, is a brevideciduous species that refoliates in the middle of the dry season (Rivera et al. 2002). I do not have leaf phenology data for *Cordia*, but it was observed to have leaves at the end of the dry season. On the other hand, *Tabebuia* had minimum TNC concentration at the end of the dry (or dormant) season. This species sheds leaves during the early dry season and refoliates only at the start of the rainy season (Borchert et al. 2002, pers. obs.). However, its fine root growth commences before the onset of the rainy season (pers. obs.). Thus, decreased TNC concentrations likely result from maintenance respiration and fine root growth during the dry season.

The temporal changes in TNC concentrations with resource availability highlight the importance of taking not only season but also development stage into consideration. All species in their first year had TNC concentrations that responded to light availability. However, by the third wet season, concentrations were not linked to light availability in most species-site combinations. In general, studies with seedlings less than two years old have found a positive relationship between TNC concentrations and light availability (e.g., Mooney et al. 1995, Naidu and DeLucia 1997, Gansert and Sprick 1998, Chapter 2), while those with older seedlings or saplings have found no correlations with light availability (e.g., Kobe 1997, Canham et al. 1999). It is possible that the lack of a relationship with light in the older seedlings was due to changes in light levels. However, the lack of a light effect in older seedlings could also reflect the 'ghost of TNC accumulations and withdrawals past'. The combination of earlier allocation to TNC and of repeated TNC withdrawals – possibly resulting from herbivory, refoliation and drought stresses – likely leads to the absence of current light effects on point measurements of root TNC concentrations in older seedlings.

Relationships among TNC, survival and growth

A trade-off between low-resource survival and high-resource growth (Kitajima 1994, Kobe et al. 1995, Lin et al. 2002, Schreeg et al. 2005) implies differences in allocation to different functions within the plants, such as allocation towards more fine root mass, or increased nutrient uptake rates to increase growth rates, or allocation towards greater defense and storage to increase survival (Kobe 1997). I had thus expected to see a trade-off between growth and allocation to TNC across species, because limited resources would have to be allocated towards either growth or survival (Chapter 3). However, a negative correlation between growth and TNC is evident only in the low P site (Carreta) where most species appear to be more limited by soil resources than by carbon (Chapter 3), as is often the case when environmental conditions are adverse (Korner 2003). The limitation on growth but not photosynthesis could lead to the negative growth-TNC relationship seen in this site as photosynthate accumulates and is stored rather than used towards new resource-harvesting structures.

An alternative mechanism by which TNC and growth rates might be inversely correlated would be if TNC withdrawals and growth rates are linked. Changes in TNC and leaf phenology are not always tightly linked in tropical forests (e.g., Newell et al. 2002, Wurth et al. 2005), although this likely varies with growth rates and nutrient availability. Slow-growing species adapted to low fertility sites depend on reserves for structural growth to a larger extent than do fast-growers from high fertility sites (Steinlein et al. 1993) although it is unclear whether this association is due to a species' inherent growth rate or effects of the environment or both. If growth rates determine the extent of withdrawal, we could expect to see a negative relationship between TNC concentrations and growth.

The positive relationship between growth rates and storage in the high P site suggests that where growth is not limited by soil resources, larger TNC stores allow greater structural growth, which in turn allow for greater photosynthetic income and more photosynthate to allocate to both storage and non-storage, leading to a positive correlation between growth and TNC stores. The positive relationship between TNC and growth at the high P site could also arise from higher carbon incomes that enable greater allocation to both storage and non-storage pools. However, the lack of a prevalent effect of whole plant mass on root TNC concentrations argues against this interpretation.

Contrary to expectation, there was not a positive correlation between TNC concentrations and survival across species. TNC pool sizes may be better predictors of

TNC available for future mobilization (Chapin et al. 1990) and thus, better predictors of survival. However, pool size also was not correlated with survival in any of the sites (data not shown). Species survival and root TNC concentrations also may be uncorrelated if root TNC stores are only a small fraction of available TNC pools. In adult trees in a moist forest, Wurth et al. (2005) estimated that belowground TNC pools represent less than 20% of total available TNC reserves. However, root TNC stores in our species likely represent a larger proportion of whole plant TNC pools than suggested by Wurth et al. (2005), who by measuring only coarse root TNC concentrations (and not main root axes), probably underestimated belowground TNC pools since larger diameter and lower order roots have higher storage (Singh and Srivastava 1986, Kosola et al. 2002).

The tendency for TNC and survival to be negatively correlated and for growth and TNC to be positively correlated under higher P suggests that species that grow bigger faster are better able to tolerate drought and herbivore pressure than those that stay small, maintain osmotic balance and/or conserve resources. Larger plants would have longer, deeper roots that would enable them to avoid severe water stress during the dry season by allowing them access to the moist subsoil (Borchert 2000). Lower susceptibility to drought in larger plants has been shown in some other tropical forests (Cao 2000, Poorter and Hayashida-Oliver 2000). The low soil resource availability in Carreta inhibits growth more than in Arboleda so that Carreta seedlings would take longer to escape size-dependent mortality, leading to the weak negative relationship between TNC concentrations and survival over the next year. However, in Arboleda, soil resources are more available and hence individuals can grow larger quicker and hence evade drought stress, leading to the tendency for TNC concentrations at the end of the second wet season to be positively correlated with survival over the following year in Arboleda.

Conclusions

There was substantial allocation to carbohydrate storage in all species studied in this seasonally dry tropical forest, with some seedlings having ~40% of root mass as TNC. Storage in first year seedlings varied with light availability but in older seedlings of most species, was influenced by soil resources alone, likely because withdrawals over the previous seasons obscured resource-TNC relationships. Two of three species showed an increase in dry-season root TNC concentrations, indicating that growth was apparently more limited than photosynthesis by the seasonal drought. Across species, growth and TNC were negatively correlated in the low fertility site and positively correlated in the high fertility site, but, contrary to expectations, TNC and survival were not correlated.

Acknowledgements

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Appendices

Appendix 1 Maximum likelihood parameter estimates and 95% support intervals, samples size (N) and coefficients of determination (r^2) for models relating root TNC with whole plant mass. Species abbreviations are as follows: AB – American beech; BC – Black cherry; BO – Black oak; RM – red maple; RO – Red oak; SM – sugar maple; WO – White oak.
Treatment abbreviations are as follows: HLHN – High light, high N; HLLN – High light, low N; LLHN – Low light, high N; LLLN – Low light, low N.

Sp.	Treatment	Model	N	R^2	*a*- 95% CI	*b* – 95% CI
AB	HLHN	y = a*(x^b)	22	0.92	0.10 (0.08, 0.12)	1.58 (1.26, 1.91)
	HLLN	y = a*(x^b)	24	0.89	0.20 (0.17, 0.22)	1.79 (1.39, 2.19)
	LLHN	y = exp(a*x) - 1	10	0.42	0.006 (0.005, 0.008)	--
	LLLN	y = a*exp(a*x) - 1	20	0.76	0.106 (-0.03, 0.22)	0.66 (0.35, 0.96)
BC	HLHN	y = a*exp(-b/x)	19	0.78	0.33 (0.15, 0.51)	1.971 (0.66, 3.28)
	HLLN	y = a*exp(-b/x)	23	0.81	0.33 (0.01, 0.66)	0.80 (0.46, 1.15)
	LL	y = a*exp(-b/x)	24	0.68	0.0001 (0.00005, 0.00015)	0.041 (0.03,0.06)
BO	HLHN	y = ax	10	0.80	0.21 (0.18, 0.23)	--
	HLLN	y = exp(a*x) - 1	10	0.86	0.17 (0.16, 0.18)	--
	LLHN	y = exp(a*x) - 1	10	0.77	0.10 (0.08, 0.11)	--
	LLLN	y = a*(x^b)	10	0.88	0.13 (0.1, 0.16)	2.07 (1.31, 2.82)
RM	HLHN	y = a*(x^b)	12	0.66	0.04 (0.03, 0.06)	0.80 (0.01, 0.04)
	HLLN	y = a*(x^b)	11	0.94	0.25 (0.08, 0.41)	1.36 (1.03, 1.70)
RO	HL	y = a*exp(-b/x)	46	0.86	2.84 (2.26, 3.42)	4.17 (3.04,5.29)
	LLHN	y = a*(x^b)	23	0.83	0.04 (0.02,0.06)	2.66 (1.84,3.50)
	LLLN	y = a*(x^b)	24	0.68	0.10 (0.70,0.12)	1.61 (1.11,2.10)
SM	HLHN	y = a*exp(-b/x)	12	0.85	0.25 (0.10, 0.40)	0.92 (0.27, 1.57)
	HLLN	y = a*exp(-b/x)	8	0.99	0.30 (0.24, 0.35)	0.85 (0.69,1.02)
	LLLN	y = exp(a*x) - 1	6	0.81	0.02 (0.01,0.02)	--
WO	HLHN	y = exp(a*x) – 1	16	0.95	0.20 (0.19, 0.22)	--
	HLLN	y = ax	22	0.97	0.33 (0.32,0.35)	--
	LLHN	y = ax	23	0.19	0.17 (0.14, 0.20)	--
	LLLN	y = ax	23	0.20	0.24 (0.19, 0.29)	--

Appendix 2 Maximum likelihood parameter estimates and 95% support intervals, samples size (N) and coefficients of determination (r^2) for models relating structural root mass with whole plant mass. Species and treatment abbreviations are as in Appendix 1.

Sp.	Treatment	Model	N	R^2	*a*- 95% CI	*b* – 95% CI
AB	HLHN	y = ax	22	0.93	0.34 (0.31, 0.36)	--
	HLLN	y = a*exp(-b/x)	24	0.94	1.04 (0.89, 1.19)	0.90 (0.77, 1.03)
	LLHN	y = ax	10	0.55	0.17 (0.15,0.19)	--
	LLLN	y = a*(x^b)	20	0.85	0.58 (0.23, 0.94)	1.61 (1.21, 2.01)
BC	HLHN	y = a*(x^b)	19	0.96	0.33 (0.26,0.41)	1.20 (1.00, 1.41)
	HLLN	y = a*(x^b)	23	0.97	0.73 (0.57, 0.88)	1.35 (1.16,1.53)
	LL	y = a*(x^b)	24	0.45	0.04 (0.007,0.07)	0.51 (0.26,0.76)
BO	HL	y = ax	20	0.76	0.38 (0.35,0.42)	--
	LL	y = ax	20	0.90	0.34 (0.32, 0.36)	--
RM	HLHN	y = a*exp(-b/x)	12	0.93	0.52 (41, 0.63)	0.71 (0.51, 0.91)
	HLLN	y = exp(a*x) – 1	11	0.90	0.35 (0.31, 0.39)	--
RO	HLHN	y = a*(x^b)	24	0.92	0.24 (0.08, 0.40)	1.27 (0.97, 1.56)
	HLLN	y = ax	22	0.85	0.38 (0.34, 0.41)	--
	LLHN	y = a*(x^b)	23	0.85	0.39 (0.35, 0.42)	0.81 (0.64, 0.98)
	LLLN	y = ax	24	0.92	0.37 (0.36, 0.39)	--
SM	HLHN	y = a*(x^b)	12	0.99	0.37 (0.35,0.40)	1.28 (1.08,1.48)
	HLLN	y = a*(x^b)	8	0.98	0.45 (0.41, 0.50)	1.27 (1.04, 1.51)
	LLLN	y = exp(a*x) – 1	6	0.81	0.19 (0.15,0.24)	--
WO	HL	y = ax	38	0.97	0.41 (0.39,0.42)	--
	HL	y = a*(x^b)	46	0.	0.37 (0.32, 0.43)	0.83 (0.46, 1.20)

Appendix 3 Maximum likelihood estimates and 95% support for parameters, sample sizes (N), coefficients of determination (r^2)and variances for the best approximating models relating surface area of fine roots (diameter < 1mm) (cm^2) and structural root mass (g). Species and treatment abbreviations are as in Appendix 1.

Species	Treatment	N	Model	a (95% support)	b (95% support)	Variance (95% support)	r^2
AB	HLHN	22	y = a*(x^b)	153.5 (128.8, 178.3)	0.59 (0.42, 0.77)	22.4 (3.1, 41.8)	0.81
	HLLN	24	y = a*(x^b)	246.9 (186.2, 307.7)	0.78 (0.54, 1.01)	22.4 (3.1, 41.8)	0.80
	LLHN & LLLN	30	y = ax	285.7 (259.4, 312.0)		2.8 (1.4, 4.0)	0.78
BC	HLHN	18	y = ax	324.6 (303.2, 346.0)		25.7 (0.25, 51.1)	0.98
	HLLN	23	y = ax	368.8 (333.2, 404.3)		10.4 (2.4, 18.3)	0.86
	LLHN & LLLN	24	y = a*(x^b)	9.0 (-9.1952, 27.3)	0.19 (-0.21, 0.60)	1.0 (0.7, 1.4)	0.05
BO	HLHN	10	y = ax	115.4 (80.0, 150.8)		87.6 (-71.7, 247.0)	0.34
	HLLN	11	y = ax	147.2 (126.6, 168.0)		34.5 (-17.8, 86.8)	0.60
	LLHN	10	y = a*(x^b)	87.8 (52.6, 123.1)	0.55 (0.19, 0.91)	6.8 (-2.7, 16.3)	0.63
	LLLN	10	y = ax	171.3 (134.1, 208.5)		17.9 (-9.0, 44.9)	0.53
RM	HLHN	12	y = a*(x^b)	228.3 (192.1, 264.6)	0.59 (0.49, 0.69)	6.9 (-1.3, 15.1)	0.96
	HLHN	11	y = ax	500.9 (279.1, 722.7)		5.6 (-0.7, 12.0)	0.75
RO	HLHN	24	y = ax	98.0 (83.0, 112.9)		87.8 (1.2, 174.4)	0.78
	HLLN	22	y = ax	150.6 (130.5, 170.6)		56.6 (0.4, 112.7)	0.80
	LLHN	23	y = ax	140.6 (125.3, 155.8)		16.8 (2.9, 30.8)	0.58
	LLLN	24	y = a*(x^b)	202.0 (160.8, 243.2)	1.35 (1.02, 1.68)	18.1 (3.0, 33.3)	0.78
SM	HLHN	12	y = a*(x^b)	130.3 (104.8, 155.7)	0.44 (0.29, 0.60)	10.9 (-3.1, 24.8)	0.87
	HLLN	9	y = a*(x^b)	190.0 (109.8,270.3)	0.58 (0.29,0,86)	16.8 (-13.8,47.4)	0.78
WO	HLHN & HLLN	38	y = ax	117.6 (105.1, 130.0)		20.1 (11.9, 28.5)	0.75
	LLHN & LLLN	46	y = ax	125.8 (111.4, 140.2)		25.8 (8.9, 42.8)	0.14

Appendix 4 Maximum likelihood estimates and 95% support for parameters, sample sizes (N), coefficients of determination (r^2)and variances for the best approximating models relating surface area of fine roots (diameter < 1mm) (cm^2) and total root mass (g). Species and treatment abbreviations are as in Appendix 1.

Species	Treatment	N	Model	a	b	Variance	r^2
				(95% support)	(95% support)	(95% support)	
AB	HLHN	22	y = a*(x^b)	122.5 (107.4, 137.6)	0.51 (0.35, 0.67)	18.7 (2.1, 35.3)	0.79
	HLLN	24	y = a*(x^b)	171.2 (144.7, 197.7)	0.66 (0.45, 0.87)	23.1 (3.1, 43.1)	0.79
	LLHN & LLLN	30	y = ax	264.6 (240.7, 288.6)		2.7 (1.42, 4.0)	0.78
BC	HLHN	19	y = ax	284.9 (262.8, 307.0)		30.2 (-0.3, 60.7)	0.97
	HLLN	24	y = a*(x^b)	207.2 (127.9, 286.6)	0.75 (0.52, 0.98)	11.3 (2.5, 20.0)	0.84
	LLHN & LLLN	24	y = a*(x^b)	9.0 (-9.1, 27.2)	0.19 (-0.21, 0.59)	1.0 (0.7, 1.4)	0.05
BO	HLHN	11	y = ax	76.3 (56.0, 96. 5)			0.37
	HLLN	11	y = ax	92.1 (78.8, 105.3)		35.3 (-18.2, 88.8)	0.57
	LLHN	12	y = a*(x^b)	74.9 (57.7, 91.9)	0.50 (0.24, 0.76)	6.5 (-1.0, 14.0)	0.68
	LLLN	11	y = a*(x^b)	88.6 (54.5, 122.7)	0.54 (-0.04, 1.12)	18.8 (-9.6, 47.1)	0.44
RM	HLHN	18	y = a*(x^b)	214.2 (183.5, 245.0)	0.63 (0.53, 0.74)	8.1 (0.9, 15.3)	0.97
	HLHN	17	y = ax	375.2 (313.1, 437.3)		5.0 (1.0, 9.1)	0.82
RO	HLHN	24	y = ax	69.2 (59.0, 79.5)		85.6 (1.4, 169.8)	0.78
	HLLN	22	y = ax	90.2 (78.4, 102.0)		55.7 (0.5, 111.0)	0.81
	LLHN & LLLN	48	y = ax	117.7 (108.0, 127.2)		20.4 (9.0, 31.7)	0.62
SM	HLHN & HLLN	21	y = a*(x^b)	127.2 (105.5, 149.4)	0.44 (0.30, 0.58)	16.2 (1.7, 30.8)	0.77
WO	HLHN	17	y = ax	66.7 (49.8, 83.6)		34.6 (-3.7, 72.8)	0.62
	HLLN	24	y = ax	66.1 (59.9, 72.3)		17.8 (3.3, 32.3)	0.86
	LLHN & LLLN	48	y = a*(x^b)	48.7 (32.4, 65.1)	0.36 (-0.01, 0.73)	13.6 (6.4, 20.8)	0.09

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