BIOMASS ALLOCATION AND GROWTH OF TROPICAL DRY FOREST TREE

SEEDLINGS ACROSS LIGHT AND SOIL GRADIENTS.

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This dissertation is dedicated to the memory of Francis R. Gates.

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ABSTRACT

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This dissertation examines the effect of abiotic factors on the growth of tropical dry forest tree seedlings, and the developmental plasticity that allows seedlings to respond to those factors. Different species of tropical dry forest tree seedling are demonstrated to reverse ranks in response on different combinations of light and soil environments that occur within the tropical dry forest. This is interpreted to indicate that tropical dry forest species may be niche-differentiated with respect to soil. This is found to conflict with the assumption of ecological equivalence central to neutral models of community structure.

Neutral models are examined further and it is determined that fitness manifolds cannot reconcile the niche-differentiation and neutral perspectives without restrictive assumptions about the distribution of environmental heterogeneity in nature.

Two experiments designed to test whether or not dry-forest tree species demonstrate adaptive plasticity in biomass allocation that is consistent with the balanced growth hypothesis have conflicting results. In the first study, individuals of the dry forest tree *Gliricidia sepium* are shown to shift their developmental allometries so that a larger fraction of biomass is allocated to leaves in high nutrient environments and a larger fraction is allocated to lateral roots in low nutrient environments, although the effect is observed only at small sizes for lateral roots. It is demonstrated that failing to account for allometry in analyses of adaptive plasticity will often lead to spurious results. It is further demonstrated that when taproot biomass is large, root:shoot ratio is a poor indicator of allocation to belowground foraging.

In a second experiment that includes the effect of both light and soil on *G. sepium* and *Hymenaea coubaril*, two species that differ greatly in life-history characteristics, soil is shown to have a negligible effect on morphology and biomass allocation while the effect of light is uniformly strong. Morphology is measured in terms of root and leaf surface area rather than as mass in individual biomass components. The magnitude of the plastic response is shown to be greater for *G. sepium* than for *H. coubaril*.

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Chapter One: Tropical dry forest tree seedlings reverse rank in

performance across light and soil gradients.

Abstract

The role of niche differentiation in structuring tropical forest tree communities is highly controversial. Neutral models assume demographic equivalence exists among species. This assumption is justified by the argument that species are constrained to occupy a manifold in niche space that is defined by a trade-off between the ability to survive in shade and the ability to respond to high light levels with increased growth rates. The regeneration niche perspective assumes that a number of ecological axes exist along which species could be differentiated as they pass through different developmental stages. In order to test whether species may be niche differentiated with respect to factors other than light, specifically differences in the soil environment, we measured rates of growth and survival of six tropical dry forest tree species across all combinations of three light and three soil environments that occur in the local community from which these species were drawn. We found that soil had a large effect on growth rate and emergence but not on post-emergence survival. Species responded individualistically to the soil gradient such that rank reversals in growth rate were observed across treatment cells. As a result, the slope of the fitness manifold was dependent on the soil environment in which it was measured. We conclude that these results are inconsistent with the idea that tropical dry forest species are ecologically equivalent in the way assumed by neutral models of community organization.

Introduction

Understanding the mechanisms that permit coexistence and determine the relative abundance of different species in natural communities is an important goal of ecological research. Niche differentiation resulting from species differences in life history traits and resource requirements has generally been considered to be the most important process in structuring communities (Hutchinson 1959, Kingsolver and Paine 1991, Tokeshi 1999, Hubbell 2001, Chase and Leibold 2003). Under such a "niche-assembly" model, environments are considered to be heterogeneous and the habitat divisible into realized niches within which each species can persist (Hutchinson 1959). The problem with this explanation is that it is difficult to imagine how niche differentiation explains species persistence in communities where species richness is high and where organisms are presumed to compete for a limited set of resources. This is the case for tree communities in the tropics. Plants exhibit much more overlap in resource requirements than do animal species, which limits the number of potential axes along which any habitat can be divided, and rainforest species richness can exceed 200 species in a 0.1 ha plot (Gentry 1988).

Current models for the maintenance of species diversity in tropical forest can be sorted into two camps. Hubbell(2001) has developed a steady-state neutral model in which species are assumed to be ecologically identical, in that the probability that a death in the community is replaced by an individual of any given species depends only on the abundance of that species in the community. This precludes niche differentiation, and local communities are predicted to exist in a non-equilibrium state with relative species

abundances shifting randomly over time (Hubbell 2001). Grubb, on the other hand, recognized that although many tree species may be ecologically equivalent as adults, a number of ecological axes exist along which species could be differentiated as they pass through life history stages (Grubb 1977). He termed this the regeneration niche of the species, and argued that differentiation in the regeneration niche could lead to coexistence. These models are not mutually exclusive and neither has logical primacy over the other.

Species are obviously not ecologically equivalent, so in order to justify the per capita demographic equivalence assumed in his model, Hubbell relies on the concept of life history manifolds. A life history manifold is an axis through niche space along which all species are constrained to be arrayed, and is defined by trade-offs in life history characteristics. Hubbell argues that the operative life history manifold in tropical forests is the well-known trade-off between relative growth rate (RGR)1q under high irradiance and survival in shade (Hubbell 2001, fig. 10.1). The result, according to Hubbell, is that "niche differentiation along life history trade-offs is the very mechanism by which per capita relative fitnesses are equalized among the coexisting species in a community" (Hubbell 2001, p. 325). Implicit in this justification, however, is the idea that the light gradient is of overriding importance in tropical forest communities such that either the effect of other environmental factors on fitness will be trivial relative to the effect of light, or that the organismal response to these secondary factors involves other trade-offs such that the form of the fitness manifold remains constant.

Although most studies of niche-differentiation in tropical forests have focused on the effects of light (see references in Brokaw and Busing 2000), a growing body of evidence suggests that variation in the soil environment has a strong effect on plant performance, and that species may respond individualistically to this variation. For example, studies conducted at La Selva biological station (Clark et al. 1998, Clark et al. 1999) and elsewhere (Condit et al. 2000) indicate that many species are non-randomly distributed along edaphic gradients. Although the mechanisms by which these edaphic biases are generated are not understood (Clark et al. 1998), it is possible that they are caused by differentiation in the regeneration niche.

If the niches of tropical forest tree species are differentiated with respect to light and soil, we would expect juvenile performance to vary across combinations of light and local edaphic factors such that pairs of coexisting species reverse in relative performance rank at some point on the local environmental parameter space. These rank reversals will generate edaphic biases and could promote species coexistence if juvenile performance corresponds to the probability that an individual of the species in question will survive to adulthood. Latham (1992) took a similar approach with temperate tree species and documented rank reversals in RGR among species in response to different combinations of light and soil nutrients. Importantly, levels of light and nutrients in this study were similar to the local environmental heterogeneity that would be encountered by seedlings in a mesic upland temperate forest.

The study examines tree seedling growth and survival in response to variables that occur on two scales: edaphic differences that occur at the mesoscale landscape and the

light conditions patchily superimposed upon them. Clark et al. (1999) define the mesoscale landscape as the scale from ~1 to 100 km^2: larger than what is investigated in most plot studies but smaller than major biogeographic regions. This is the scale at which Clark et al. (1998, 1999) determined that edaphic biases were common at La Selva. Most conserved wildlands fall within this classification. Rather than applying nutrient treatments to plants grown in the greenhouse, seedlings were grown in transplanted, field-collected soils in common gardens situated in a range of light conditions in the field. This is preferable to greenhouse studies because only the ranges (and covariances) of environmental variables that actually occur within a community are relevant to the question of coexistence and relative abundance in that community.

Obviously, dispersal limitation and stochastic factors will often prevent species from occupying the sites at which they perform best (Hurtt and Pacala 1995), so the idea that communities are either niche- or neutrally assembled is in fact a false dichotomy. The question of interest is to determine the relative strength of each process in nature. In fact, Hubbell has employed a mixed-model approach to describe systems where clear edaphic associations occur (Terborgh et al. 1996, Hubbell 2001).

If Tropical dry forest (TDF) soils differentiate the niche space of tree seedlings, we would expect to see strong effects of soil on seedling performance parameters such as emergence, survival, and growth and we would expect species the performance rankings of the species to change under different combinations of the light and soil environments. In particular, we would expect rank reversals to occur with respect to soil treatments within common light environments. Finally, if Hubbell's hypothesis that niche

differentiation leads to ecological equivalence is correct, we expect that either species that respond to some soil environments with increased growth in sun suffer an offsetting increased rate of mortality in the shade in those same environments, or that that all species gain equally from all soil environments.

Methods

Species and site

The study was conducted from June to November 2001 in Sector Santa Rosa of the Area de Conservación Guanacaste (ACG), a 110,000 hectare conserved wildland in northwestern Costa Rica. Sector Santa Rosa is characterized by remnant patches of deciduous and semi-deciduous forest of 0-400 years of age ((Janzen 1986)). Annual precipitation averages 1500 mm a year with most falling between late May and early December, with a two week break in August.

Close to 200 tree species are present in the 30,000 hectares of tropical dry forest (TDF) of Santa Rosa (Janzen and Liesner 1980). The species included in this experiment are or were historically common components of TDF. Six legumes (Fabaceae) were chosen based on seed availability and to represent the range of life-histories of TDF trees: from small-seeded, heliophytic early colonizers to large-seeded, slow-growing, mature-phase dominants (Table 1). Of these, only *H. coubaril* did not nodulate.

Soil collection and characterization

The three clay loam soils used were provisionally categorized as poor, intermediate, and high quality based on previous observations that native tree seedlings

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grown in them exhibited considerable differences in growth rate (Klemens unpublished data). Preliminary analyses indicate that soils differed most in pH, organic matter content, and cation exchange capacity. Poor soil was collected from recently burned early-successional scrub alongside a firebreak in the upland section of Santa Rosa. Intermediate soil was collected from the approximately 30 year old regeneration surrounding the administration area of sector Santa Rosa. Both of these soils formed on the Santa Rosa plateau, which is Pleistocene volcanic ignimbrite of the Bagaces formation (Castillo Muñoz 1993). High quality soils were collected near a road cut in the lowland between the Santa Rosa plateau and the Pacific Ocean, near the Río Poza Salada. This lowland clay loam is more friable than the other soils, possibly due to the clay fraction containing less smectite (2:1) clays (pers. obs.), and is formed of Quaternary alluvial deposits with outcroppings of organic limestone (Castillo Muñoz 1993). No detailed soil map exists for Santa Rosa. In all three soils, the horizons were poorly defined and no diagnostic horizons were present, so all three were classified as Ustepts (USDA 1998). Only the top 30-40 cm of soil was collected.

Poor and high quality soils were each collected and transported by truck in five 3-4 m^3 batches and deposited on polyethylene sheeting in order to avoid contact with the local soil. Soils were mixed by hand within batches. Intermediate soil was mixed in smaller batches as it was transported by wheelbarrow. Due to the extremely large volume of soil involved, batches were bagged one at a time and intermediate quality soil was bagged once a soil pile equivalent to one 3-4m^3 batch had accumulated. Soils were packed into black polyethylene nursery bags approximately 16 cm in diameter and 24 cm

tall; bags were drained by 22 holes (0.75 mm diameter) 18 on the sides and 4 on the bottom. Filled bags from each batch were then distributed evenly among the fifteen experimental sites.

Experimental sites/ light treatments

Fifteen 6m by 6m experimental sites were chosen such that there were five replicate sites representing one of three light conditions typical of second-growth tropical dry forest: closed canopy, canopy gap, and open field. All sites were located within a 500 m radius. Each site was surrounded by a 180 cm tall wire fence to exclude mammalian herbivores and a second fence 30cm outside the first to exclude leaf-cutter ants. This fence consisted of 45cm of black polyethylene buried 5cm deep and folded over a wire at the top. Herbaceous vegetation within the fence was clipped and leaves were removed from shrubs and saplings located in the site in order to increase the homogeneity of the light environment within each site.

The 480 soil bags (160 of each soil type) were arranged in 8 3 X 20 rows within each site, with each 3-bag row of the block containing one each of the three soil types. Plants from three of these rows were randomly selected to be harvested for this experiment (see below) and the remaining five were reserved for harvest as part of a concurrent study of biomass allocation patterns (chapter 4). Rows were for maintenance and not designed to be statistical blocks, as they did not capture within plot spatial variation.

To determine if light levels within each treatment were more similar to one another than to the other treatments, the percent of above canopy irradiance reaching

plant level at the center of the site was measured once before planting and three times over the course of the experiment. Digital hemispherical photographs were taken at 75 cm above the ground with a Nikon Coolpix 990 with Nikon FC-E8 fisheye lens adapter at a resolution of 2048 X 1536 pixels. Photographs were taken between 5 and 6 AM or 5 and 6 PM, or on completely overcast days. Adobe Photoshop 5.0 was used to produce a high contrast black and white image. The image was then thresholded and analyzed using the Gap Light Analyzer program (Frazer et al. 1999).

As radiation data were not available for Santa Rosa, 50% contribution of direct and diffuse radiation was assumed for the above canopy total. The percentage of total radiation penetrating the canopy was calculated using the Universal Overcast Sky model; topography was deemed to be unimportant at these sites. Light measurements were made one day after planting, 40 days after planting, and during the time of harvest (157 days after planting). To obtain growing season averages, it was assumed that differences in penetration changed linearly with time between observations and a weighted average was caculated for each site. Measurements were not evenly spaced in time because in dry forest change in canopy cover is much more rapid in the early portion of the growing season.

Planting

Seeds were planted from 2-4 June 2001, one species per bag. Two seeds were planted in each bag and later thinned to one for all species except H. coubaril, which was planted one seed per bag. Wind dispersed *G. sepium*, *L. felipei*, and *D. retusa* seeds were planted flat on their side, just below the soil surface, *S. saman* and *E. cyclocarpum* were

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planted approximately 7.5 mm below the soil surface and , and larger *H. coubaril* seeds 10-15mm below the surface. Seeds of the three animal dispersed species were scarified with a file.

Watering

All soil bags were watered to saturation for two days after planting. Due to extremely dry weather (67 mm rainfall between 10 June and 31 July, compared to 202 ± 111 mm (average $\pm$ 1 SD) during the same period for all other years from 1994 to 2002), it was necessary to water the soil bags from shortly after planting until early August. In an attempt to break natural covariation between radiation and soil moisture due to the drought, the frequency of watering was varied among light treatments. Approximately100 ml of water (the equivalent of 5mm rainfall) was applied to each bag: every two weeks in shade, 2-3 times per week in canopy gap, and 3-4 times per week in full sun. Still, it was obvious that soil bags in shade treatments retained more moisture than soil bags in gap treatments, and that soil bags in gap treatments retained more moisture than soil bags in full sun.

Selection / exclusion of plants

Plants were selected randomly for harvest. Plants that had suffered significant insect or mechanical damage, or that exhibited developmental abnormalities, were not harvested. Significant damage was defined as loss of > 25% of leaf area or any damage to the apical meristem at any point during the experiment. An exception to this rule was made in the case of *D. retusa*, because cotyledons were seriously damaged by a Chrysomelid beetle immediately upon emergence. All surviving individuals were

harvested at the end of the experiment and only individuals which were deemed to have been significantly damaged later in the experiment were excluded. A great deal of insect damage was prevented by regularly searching for and removing insect larvae by hand. Many sawfly and macrolepidopteran larvae were removed before they caused significant damage.

Data collection

Most plants (n=1281) were harvested between 1 and 21 November, with a few (n=135) harvested 26 November. The stem was cut at the soil surface, and leaf number, stem height, and stem diameter at 5 cm above the soil surface were recorded. The number and total length of all secondary and tertiary branches was measured. Leaves and stems were dried separately at 70° C for 5-7 days, then cooled and stored in plastic bags to prevent them from taking on atmospheric water before weighing.

Relative growth rate (RGR) was calculated for shoots only. Starting dry biomass was determined by assigning half of the biomass of a newly germinated seedling to shoots. To calculate seedling mass, seeds were sprouted in sunlight in wet tissue paper until the primary radicle emerged, and the seed coat was removed and discarded before drying.

Emergence and survival rates were calculated for each species in each soil and site combination. Emergence was calculated as the percentage of planted seeds that successfully opened their cotyledons (or unfolded the first leaf in the case of *L. felipei* which germinates hypogeally). Two species showed very low emergence rates that were determined to be due to outside factors (early herbivory on *D. retusa* that may have killed

individuals before they could be counted, and overly vigorous scarification in the case of

S. saman) and were excluded from that analysis so as not to bias results. Survival was

calculated as the percentage of individuals that successfully emerged that were alive at

the time of harvest (excluding those that were thinned from bags in which more than one

seed emerged).

Data analysis

To determine the relative effect of light, soil, and species identity on growth rate,

a three-way ANOVA was conducted with RGR as the response variable, light treatment,

soil type, and species as main effects, and site as a random effect nested within light.

RGR was natural log transformed prior to analysis to improve homogeneity of cell

variances. Due to a number of empty cells resulting from low survival within the shade

treatment, shade plots were excluded from the three-way ANOVA and only included in

the within-plot comparisons. Post-hoc comparisons between all possible pairwise

combinations of species within all light X soil combinations were made using a Tukey-

Kramer HSD test with alpha = 0.05.

Data showed significant departures from the assumptions of homogeneity of

variances and of normality, even after transformation. For comparisons within treatment

cells, however, distribution of residuals approached normality and heterogeneity of

variances was much reduced. For the factorial comparison, data were rank transformed

and a three-way ANOVA was performed on the ranks. The results differed only slightly

from the parametric ANOVA, as noted below. Similarly, for within-cell comparisons,

data were ranked and a Kruskal-Wallis test was performed on the ranks, and results did

not differ from the parametric analysis. Therefore 95% confidence intervals computed from the unranked data are presented. All statistical tests were performed with JMP (SAS 1997).

Similarly, to measure the effects of light, soil, and species identity on emergence and survival two three-way ANOVAs were performed with survival and emergence as the response variables. As these variables were measured at the plot level, the factor site was not included in the analyses. For survival, residuals of the ANOVA were approximately normally distributed and for emergence the distribution of residuals did not differ from normality. Because soil was shown to have no effect on survival, 95% confidence intervals were calculated within light treatments only, with values averaged over all soil treatments. For emergence, 95% confidence intervals are presented for all light X soil combinations.

In order to test for a tradeoff between high growth rate in light and survival in low light among these species, and to assess the effect of soil on the form of Hubbell's proposed fitness manifold, mean RGRs in open and gap sites for each species were regressed (separately) on survival in shade sites. The regressions were performed once with data pooled across soils, and also separately for each soil.

Although data on seedling height, stem diameter, and leaf number were collected, these data had a complex relationship to light and soil treatments due to species-specific morphological responses, and were impossible to compare across species. Morphological data collected on a subset of these species that explicitly considers growth allometries

and developmental plasticity will be presented elsewhere (Klemens, manuscript), and not considered here.

Results

Light environments

Canopy analysis revealed that for the most part different sites within light treatments allowed similar light penetration. Values ranged from 80-95% of above-canopy radiation for the four open treatments harvested, 41-54% for four of the gap treatments, with a fifth site at 32%, and 10-20% for four of the shade treatments, with a fifth site at 32%. The outlying gap and shade plots were excluded from the within treatment cell comparisons of species means for survival rate and RGR, but not from comparisons of emergence rate as penetration values did group by treatment at the beginning of the experiment. The three-way ANOVAs for RGR and survival rate were performed two ways: with the gap outlier included and with it excluded (shade plants were not included in the three-way ANOVA), and as there was no difference in the results of the analysis, the ANOVA results presented below include the outlying plot.

Effects of light and soil on RGR

Results of the 3-way ANOVA show that all sources of variation except for Site had affected RGR (Table 2). Of particular interest are the significant Species X Light and Species X Soil interactions, indicating that the species responded differently to the light and soil treatments applied. Mean RGR for each Species X Light X Soil combination are presented with 95% confidence intervals in Figure 1. Species generally responded to

increased irradiance with increased growth, with the exception of *S. saman* and *E. cyclocarpum*, which showed no difference or a slight decrease in RGR between gap and open light treatments. In general species showed a weak response to the intermediate quality soil, and fell into three groups in terms of their response to high quality soil: three species responded to the high quality soil with an increase in RGR in gap and open light treatments (*G. sepium*, *S. saman*, and *E. cyclocarpum*), two species responded to the high quality soil only in open treatments (*L. felipei* and *D. retusa*), and *H. coubaril* showed little response to soil in any treatment (Figure 1). Interestingly the MS values indicate that the main effect Soil explained a much higher proportion of the total variance in RGR than did Light (Table 2).

Several changes in species rank in RGR in response to light and soil treatment were apparent. For example, *H. coubaril* is the second highest ranked species in poor and intermediate quality soil in open light, an increase from its low rank in the same soils in gap light, but lower than its last place rank in high quality soil in open light. At the opposite extreme, *S. saman* showed a large enough response to high quality soil to move it to the upper tier of species in performance rank, but showed little response to light (Figure 1).

Emergence

For emergence rate, all main effects and the Species X Light and Species X Soil interactions were significant (Table 3). Average emergence rate for each Species X Light X Soil combination are presented with 95% confidence intervals in Figure 2. For three of the four species considered, emergence rates tended to increase with irradiance and to be

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higher in intermediate quality soil. This trend was most pronounced for *G. sepium*. *H. coubaril* did not appear to be sensitive to changes in light treatment or soil quality. Because of low replication, 95% confidence intervals were wide and no rank reversals were observable.

Survival

Post-emergence survival rate was affected only by the main effects of Species and Light, and the Species X Light interaction (Table 4). Average survival rates for each Species X Light combination are presented with 95% confidence intervals in (Figure 3). Survival rate was uniformly high and tended to increase between shade and open light for four species, with *H. coubaril* again showing the flattest response. *D. retusa* showed uniformly poor survival in all treatments, perhaps due to continued herbivory on apical meristems that went undetected (due to the rapidity with which meristem herbivory on *D. retusa* occurred, as described above). When the ANOVA was conducted with *D. retusa* data excluded, the results were the same. *G. sepium* showed the strongest response to light treatment, exhibiting low and highly variable survival in shade light nearly complete survival in high light. *G. sepium* seedlings in shade treatments were highly etiolated compared to seedlings in the other light treatments (Klemens unpublished data) and many seedlings were killed by being knocked over by heavy rains soon after emergence. The wide confidence intervals for all species in the shade treatments were in part the result of smaller sample sizes in shade due to differential emergence rates (Figure 2).

Trade-off between RGR and survival

Parameters from the regressions of RGR in gap and open light treatments on survival in shade are presented in Table 5. Significant regressions were attained only for poor and high quality soils in open light (Figure 4). Due to the small differences observed between survival rates for most species (Figure 3), the regressions were largely driven by the growth response of *G. sepium*, which had the lowest survival rate in shade and the highest RGR in open and gap light. Consequently, the trend observed was for the relationship to have a steeper slope in higher quality soil. Comparing the slope of the two significant regressions reveals that in high quality soil the slope exceeded that observed in the low quality soil by a factor of four (Table 5).

Discussion

This experiment demonstrated clear differences in the way that seedlings of different species of TDF trees respond to gradients in light availability and soil quality. These gradients are representative of the observed range of variation in these factors over a Costa Rican TDF mesoscale landscape. If the measures of performance considered here correspond to an individual's probability of reaching reproductive maturity, then the observed differential performance among species will lead to edaphic biases in the distributions of adult trees so long as dispersal limitation does not prevent species from reaching favored sites. This is particularly clear in those cases where rank reversals in species performance occurred. Since rank reversals occurred within the range of environmental heterogeneity present on this landscape, it provides evidence that

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differentiation in the regeneration niche with respect to soils may be a mechanism

mediating species coexistence in this forest.

The idea that niche differentiation along a single fitness manifold has resulted in

ecological equivalence among these species is not supported by these data. As soil had a

large but differential effect on growth and no detectable effect on survival, the slope of

the proposed fitness manifold differed across substrates (Figure 4). Given the large effect

of soil on a subset of the species, it is hard to see how average fitness values for different

species could be equivalent at the landscape scale without making restrictive assumptions

about the spatial distribution of soils across that landscape.

The idea of the fitness manifold is also not supported by other characteristics of

the species studied. For fitness manifolds to result in ecological equivalence, there must

be a covariance of species traits such that all species can be categorized as falling along a

gradient from small-seeded, fast-growing, precocious, widely dispersing, poorly

defended, and short-lived to large-seeded, slow-growing, late-maturing, dispersal limited,

well-defended, and long-lived. Or, to put it another way, to return to the idea that species

all well described as falling along a continuum from r- to K- selected. Clearly this is not

the case in this system. Fast growing *G. sepium* disperses its seeds over short distances

and may be a very large, long-lived tree. Despite differences in seed size and phenology,

P. saman and *E. cyclocarpum* are both long-lived trees with very similar adult

morphologies. In two of three soils, *H. coubaril* was the second fastest grower under high

light despite being a classic late-successional large-seeded dispersal-limited species. In an

exemplary study, Clark and Clark (1992) showed that when growth and survival of

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tropical trees was considered at multiple life stages, suites of traits were not so correlated as they would seem to be from examining only a single size class, and found that the observed relationship of seedling traits to juvenile and adult performance across environmental gradients was highly complex.

Rank reversals in emergence and survival rates with respect to soil were not observed in this study, although both were strongly affected by environmental heterogeneity. Although the effect of the Soil X Species interaction on emergence was significant the data does not indicate any trade-off between emergence and growth across soils (Figure 2). Emergence rates were undoubtedly inflated from what they would be in nature as pre-treatment, planting, and intitial watering of seeds were designed to provide optimal germination conditions. Post-emergence survival rate may also have been inflated by herbivore removal. It must also be noted that if, as seems probable, a large percentage of seedling mortality in TDF occurs during the dry season, and if over-dry-season survival is size-dependent, then seedling survival will be as or more dependent on RGR than on post-emergence pre-dry-season mortality. Even if this is the case, it would not strongly affect our interpretation of the fitness manifold, because soil had a negligible effect on growth rate in the shade (Figure 1). A study of the timing and size-dependence of mortality in naturally occurring seedlings of the species studied here is ongoing (Klemens, unpublished data).

Even though the heterogeneity considered in this experiment was constrained to only two axes of environmental variation, performance differences between species were quite clear. Furthermore, the gradient in soil quality was probably "short" relative to the

range of soil types over which these species occur in nature. Differentiation along light gradients has been well demonstrated (e.g. Kobe 1999), but there are many more ecological axes along which species can differentiate in the regeneration niche that have been less well explored (Grubb 1977). In TDF, for example, edaphic biases in tree distributions have been demonstrated across water availability gradients (Borchert 1994) and can be easily observed for many species along other gradients: for example the preferential occurrence on some species on rare substrate types such as limestone outcrops or cliff edges (Klemens pers. obs.). That the distribution of many TDF species may be strongly affected by herbivores has also been well-documented (Janzen 1970, Sullivan 2000).

Another axis of differentiation may be dispersal limitation itself, which Grubb considered part of the regeneration niche (Grubb 1977). Dispersal limitation preventing species from occupying favorable sites is undoubtedly an important factor but it is sometimes forgotten that once a species has arrived at a favorable site, dispersal limitation may reinforce edaphic biases by concentrating propagules in a favorable environment (Tilman 1994, Snyder and Chesson 2003). This should be especially true for edaphic gradients at the mesoscale landscape, where operative environmental variables are likely to be spatially autocorrelated at scales greater than the dispersal distances of most tree species (Brown 1984).

It would be very useful to understand how the distributions of adult trees in Santa Rosa conform to these identified edaphic gradients. Unfortuantely, due to the scarcity of undisturbed old-growth forest on this landscape (Janzen 1986, Janzen 1988), it would be

very difficult to demonstrate edaphic biases in this system. There is evidence of distributional biases at the landscape scale for some of the more common species, but for most species, the highly complex temporal pattern of regeneration in Santa Rosa will make it daunting if not impossible to separate habitat preference from historical effect.

Despite high replication, the analyses conducted in this experiment were plagued by high within-treatment variation. This was in part the result of attempting to make the experiment more "realistic" by using field collected soils and naturally occurring light conditions as treatment levels. As a result, some rank reversals may have gone undetected. It is not clear how to solve these problems in future studies of this type without massively increasing replication.

As for the question of whether equilibrium or neutral models best describe patterns of biodiversity in TDF, this study demonstrates that there is at least the potential for these species to be niche differentiated with respect to soil and casts doubt on the idea that a fitness manifold results in different species having equivalent fitness over this landscape. Some species in this community may still be "ecologically equivalent", due to convergent evolution or strong dispersal limitation. Among these species, population dynamics may be effectively neutral. These results highlight the importance of considering ecological axes other than the light gap - understory continuum if we are to understand the relative strength of niche differentiation in structuring tropical forest tree communities.

Tables

Table 1. Natural history characteristics of species included in the study, ranked by seed size.

Species	Seed mass (g)	Dispersal	Phenology*	Description
Gliricidia sepium	0.121	Explosion	Deciduous	Small, fast colonizer of pastures, can be large in forest. Can reproduce at 2 yrs.
Dalbergia retusa	0.133	Wind	Adults evergreen	Large tree, very hard wood, few remain, natural history not well known.
Samanea saman (Pithecellobium saman)	0.172	Animal	Adults evergreen	Large spreading tree in pastures and on riversides.
Lonchocarpus felipei	0.283	Wind	Deciduous	Medium size tree in regenerating forest and along riversides.
Enterolobium cyclocarpum	0.921	Animal	Deciduous	Large spreading tree in pastures.
Hymnaea coubaril	3.939	Animal	Evergreen	Very large tree dominant in mature TDF on wetter sites.

* Deciduous trees are leafless through the dry season, Evergreen trees replace their leaf crop once a year but maintain leaves throughout the dry season.

Table 2. Three-way ANOVA of RGR. See text for details.

Source of variation	df	Error term	MS	F	P	
Species	5	species x site (within light)	0.00068	19.12	< 0.0001	****
Light	1	site (within light)	0.00069	15.24	0.0056	**
Soil	2	soil x site (within light)	0.00117	87.85	< 0.0001	****
Species x Light	5	species x site (within light)	0.00025	6.96	0.0001	***
Species x Soil	10	species x soil x site (within light)	0.00011	16.49	< 0.0001	****
Light x Soil	2	soil x site (within light)	0.00024	18.15	< 0.0001	****
Species x Light x Soil	10	species x soil x site (within light)	0.00003	4.13	< 0.0001	****
Site (within light)	7	residual	0.00005	1.27	0.2873	
Species x Site (within light)	35	residual	0.00004	5.96	< 0.0001	****
Soil x Site (within light)	14	residual	0.00001	2.23	0.0057	**
Residual	908					

** P < .01, *** P < .001, **** P < .0001

Table 3. Three-way ANOVA of emergence. See text for details.

Source of variation	df	Error term	MS	F	P
Species	3	residual	0.2310	17.610	< 0.0001 ****
Light	2	residual	0.6729	51.299	< 0.0001 ****
Soil	2	residual	0.3218	24.531	< 0.0001 ****
Species x Light	6	species x light x soil	0.0865	6.598	< 0.0001 ****
Species x Soil	6	species x light x soil	0.0691	5.267	< 0.0001 ****
Light x Soil	4	species x light x soil	0.0295	2.249	0.0667
Species x Light x Soil	12	residual	0.0056	0.425	0.9514
Residual	144		0.0131		

**** P < .0001

Table 4. Three-way ANOVA of post-emergence survival. See text for details.

Source of variation	df	Error term	MS	F	P
Species	5	residual	0.4253	8.827	< 0.0001 ****
Light	2	residual	1.1812	24.516	< 0.0001 ****
Soil	2	residual	0.0053	0.111	0.8950
Species x Light	10	species x light x soil	0.2391	4.963	< 0.0001 ****
Species x Soil	10	species x light x soil	0.0239	0.496	0.8905
Light x Soil	4	species x light x soil	0.0139	0.289	0.8845
Species x Light x Soil	20	residual	0.0324	0.673	0.8478
Residual	216		0.0481		

**** P < .0001

Table 5. Parameters from regressions of RGR in gap and open light

conditions on seedling survival in shade.

Light	Soil	r^2	slope	pvalue
gap	poor	0.2504	-17.18	0.3120
gap	intermediate	0.2871	-22.93	0.2732
gap	high	0.1470	-33.90	0.4531
	pooled	0.2243	-24.67	0.3427
open	poor	0.4363	-39.75	0.0469 *
open	intermediate	0.4308	-56.33	0.1569
open	high	0.7277	-161.55	0.0308 *
	pooled	0.6544	-85.88	0.0513

* P < .05

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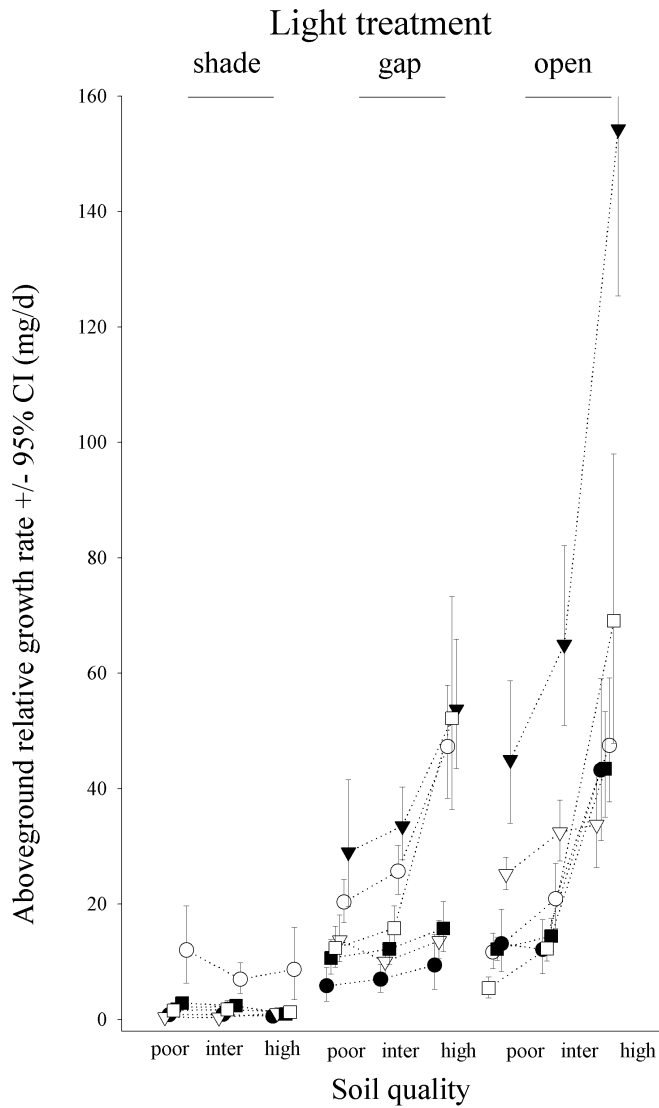
Figure 1. Treatment effects on growth rates. Symbols represent means for each combination of light and soil for each species. Species are *G. sepium* (▼), *D. retusa* (●), *S. saman* (□), *L. felipei* (■), *E. cyclocarpum* (○), and *H. coubaril* (▽).Error bars represent 95% confidence intervals.
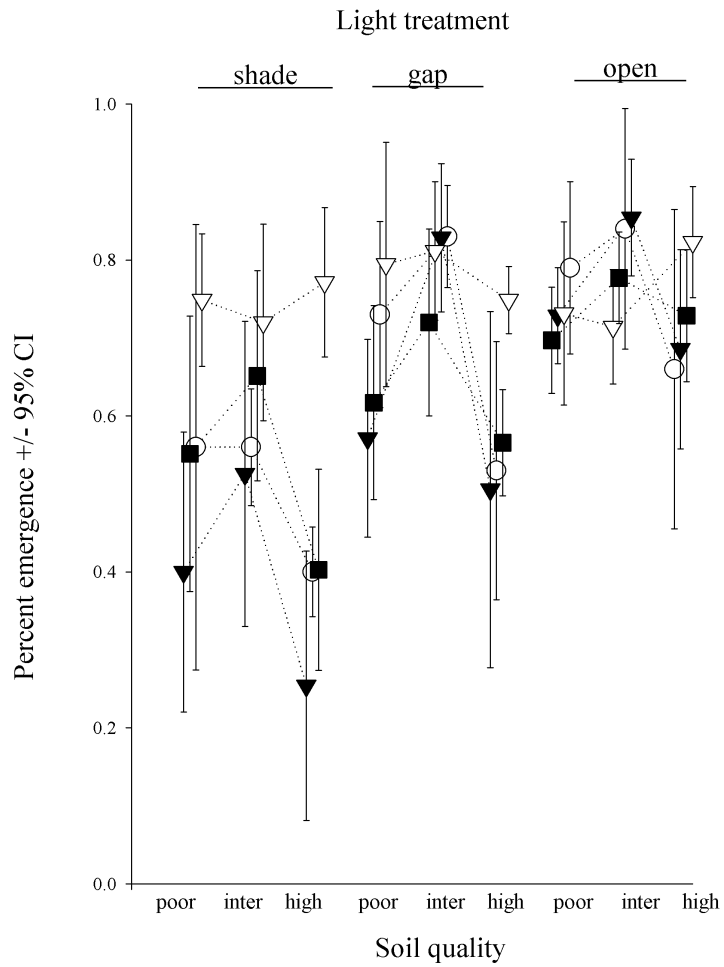
Figure 2. Treatment effects on emergence. Symbols represent the mean of the site level emergence rate for each combination of light and soil for each species. Species as in figure 1. Error bars represent 95% confidence intervals.
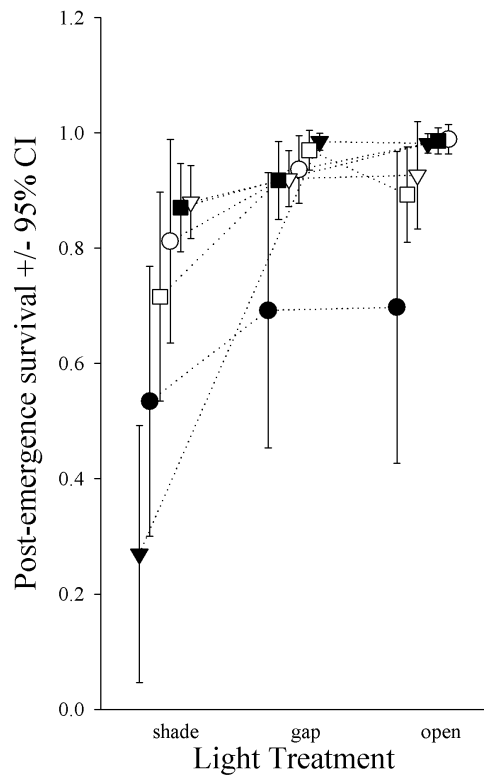
Figure 3. Treatment effects on post-emergence survival. Symbols represent the mean of the site level survival rate for each light treatment. Species as in figure 1. Error bars represent 95% confidence intervals.
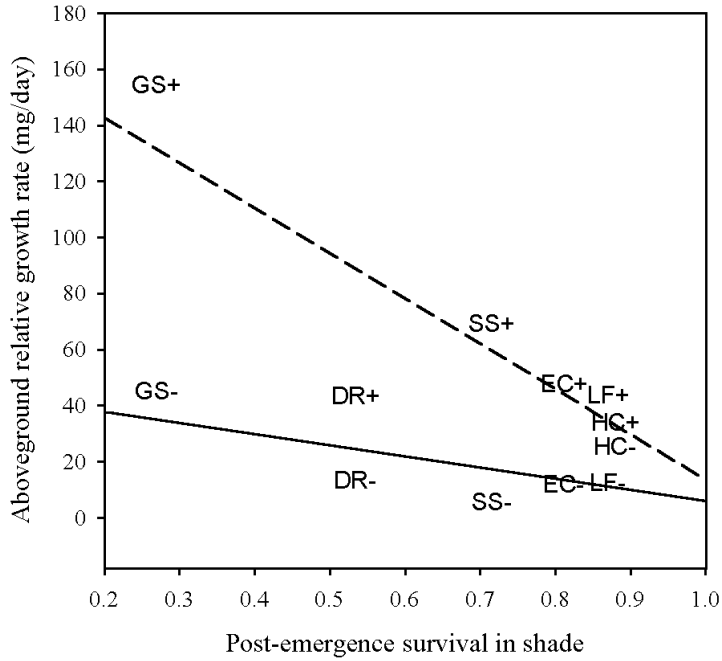
Figure 4. The fitness manifolds defined by the relationship between post-emergence survival in shade and RGR in the open light treatment. Letters are genus-species names, (+) and (-) and the dashed and solid lines indicate means and linear regressions from high and low nutrient soils, respectively.

Chapter Two: Fitness manifolds do not reconcile niche and neutral theory.

Niche differentiation, the idea that species coexistence depends on differences in organismal traits that preclude competitive dominance by any one species, is a central concept in ecology(Hutchinson 1959, Kingsolver and Paine 1991, Tokeshi 1999). It provides the basis for developing predictive theories about the distribution and abundance of organisms in nature based on an understanding of traits and how they mediate the interaction between the organism and its environment. When these theories are empirically supported, they can be expressed as assembly rules for particular biological communities (Weiher and Keddy 1999).

Stephen Hubbell (2001) has developed a model that he calls the unified neutral theory (UNT) that demonstrates that niche differentiation is not necessary for species coexistence over long intervals. This model assumes that species are ecologically equivalent, meaning that on a per-capita basis, birth and death rates are identical for all individuals in the community. Communities in this model are thus predicted to exist in a non-equilibrium state with relative species abundances shifting over time, and nature is assumed to be homogeneous. Despite these assumptions, the UNT provides a good fit to species accumulation curves and distributions of relative species abundances in certain kinds of communities, and specifically the tropical forest tree communities for which the model was first developed. Neutral theory provides the basis for predictive theories about community organization that are based on the belief that ecologists can predict community structure without explicitly considering the role of organismal traits.

Although they are often presented as being in opposition (Bell 2001, Whitfield 2002), niche and neutral theory are not mutually exclusive. Most communities likely consist of a mix of species which are maintained by some form of niche differentiation and some which are ecologically equivalent and maintained by random processes (see Terborgh et al. 1996, and the response in Hubbell 2001 Chapter 10).

Neither is a predictive theory, although predictive theories can be developed from them. Both are in a sense tautologies. Niche theory can be boiled down to the statement that organisms don't occur where they can't. Neutral theory is likewise a logical statement, albeit a mathematically complex one. Because they are independent logical statements and not mutually exclusive, there is no critical test between them. The important comparison becomes that between the assumptions of the various models and how well these assumptions apply to the observed properties of the systems of interest (Hubbell 2001).

Although most criticisms so far have focused on the fit of the model to data (McGill 2003), or the implications of variation in model assumptions on fits to data (Yu et al. 1998, Ricklefs 2003), the assumption of ecological equivalence between species is undoubtedly the most controversial feature of the UNT. The fact that species are non-randomly assembled along environmental gradients has been repeatedly demonstrated by plant ecologists over the last 100 years (Whittaker 1956) would seem to argue that the assumption of ecological equivalence is a poor one. Hubbell contends that this is not necessarily the case, and dedicates the last chapter of his monograph to reconciling the

assumption of ecological equivalence with the observation that organisms do differ in ecologically relevant ways.

Hubbell points out that among tropical forest trees there exists a well-documented trade-off between the ability to survive in deep shade and the ability to exploit high irradiance with rapid growth. Hubbell argues that this trade-off represents a fitness manifold for species, meaning an attractor in trait space along which all species will have equal fitness. In other words, all species are restricted to a set of strategies that is limited by a constraint that is a consequence of plant physiology. From this, Hubbell argues that niche differentiation is limited to the sorting out of species along the manifold. Therefore, niche differentiation will actually result in ecological equivalence as defined by the UNT, or, in Hubbell's words "*niche differentiation along life history trade-offs is the very mechanism by which per capita relative fitnesses are equalized among the coexisting species in a community*" (p.325, emphasis in original).

To support this argument, Hubbell adduces evidence assembled by Peter Reich that several plant traits tend to converge on a limited number of solutions governed by simple trade-offs (Reich et al. 1997, Reich et al. 1998, Reich et al. 1999). The resulting correlations are indeed strong and general across a wide range of biomes. It is not clear why the fact that many plant traits are governed by simple trade-offs would necessarily lead to ecological equivalence among species, however, unless one were willing to infer that *all* plant traits are similarly constrained. To take this to its logical conclusion, the result is a predicted covariance between organismal traits that takes ecology back to the idea that species can be classified on a gradient from r- to k- selected. This is also a

strong statement about the power of convergent evolution to drive all species on the landscape to a common fitness optimum, and downplays the role of evolutionary history and lineage specific constraints.

That said, the idea that fitness manifolds will promote ecological equivalence has two major problems. First, the available empirical evidence suggests that even in tropical forests the performance of plant species across environmental gradients is much more complex than would be required if fitnesses were constrained along a single axis in environmental space. Most studies of tropical forest tree communities have focused on the effects of light. Despite the fact that they have received much less attention, factors such as water or nutrient availability in the soil are likely to be important as well. The data I present in chapter 1 of this dissertation demonstrates that, in the tropical dry forest of northwest Costa Rica, soil has a large effect on the growth rates of tree seedlings (Klemens MS). However, because soil has a large effect on growth, but no effect on survival in shade, the 'fitness manifold' defined by the tradeoff between growth in high light and survival in shade changes form across soil types in a way that is inconsistent with ecological equivalence.

Numerous other studies of tropical forest trees have indicated that abiotic factors other than light play a role important in structuring tree communities either by demonstrating that tropical forest species differ in their responsiveness to nutrient levels (Gunatilleke et al. 1996, Gunatilleke et al. 1997, Gunatilleke et al. 1998) and water availability (Borchert 1994) or by documenting edaphic biases in the distributions of tropical trees (Clark et al. 1998, Clark et al. 1999, Condit et al. 2000). In addition, Clark

and Clark (1992) studied individuals of a group of tropical tree species at various life stages, and showed that the diversity of life-history strategies were not well described by a single axis.

Secondly, if environmental heterogeneity is considered explicitly, fitness manifolds will result in equivalent fitness among species in a local community only under very restrictive assumptions about the distribution of environmental variables in space. Consider the very simple example where the environments in a particular community can be divided into two bins, *a* and *b*. Let l_n be the fitness of any species at the community level, which in Hubbell's model will be 1 for all species because of the assumption of neutrality. For ecological equivalence to hold, there must exist a fitness manifold expressed as a linear tradeoff between the ability to perform in bin *a* and bin *b*, such that the average fitness across bins is constrained to equal 1 (eq. 1).

$$\lambda_n = 1 = \frac{\lambda_a + \lambda_b}{2} \qquad \text{equation 1}$$

This model fails to consider the distribution of environments *a* and *b* within the community, however. Let D_a and D_b represent the proportion of sites in the community represented by bin *a* and bin *b*. As species in the neutral model arrive at sites randomly, fitness in a particular bin must be weighted by that bin's relative availability at the community level, and l_n must be the weighted sum of bin-specific fitness (eq. 2).

$$\lambda_n = 1 = \lambda_a D_a + \lambda_b D_b \qquad \text{equation 2}$$

Now consider a matrix of species' strategies as constrained by the fitness manifold. The vector of species-specific fitnesses at the community level will be the product of the matrix of available species strategies and a vector consisting of the relative abundances of the different bins in the community. If fitness are to be equivalent at the community level, it is obvious that all values in the vector of relative abundances must be equal, in our case, sites must be evenly divided between habitat types (eq. 3). The two bin case is presented for simplicity, however, as long as fitness between bins is governed by a linear tradeoff, it will be true for any number of bins that all D values will be constrained to be $1/x$, where x is the maximum number of bins. Therefore, ecological equivalence at the community level will only obtain when environmental heterogeneity is distributed evenly across the landscape.

$$
\begin{array}{cc}
\lambda_a & \lambda_b \\
\end{array} \qquad\qquad \lambda_n
$$

$$
\begin{bmatrix}
2 & 0 \\
1.9 & 0.1 \\
\vdots & \vdots \\
1.0 & 1.0 \\
\vdots & \vdots \\
0.1 & 1.9 \\
0 & 2
\end{bmatrix}
\bullet
\begin{bmatrix}
D_a \\
D_b
\end{bmatrix}
=
\begin{bmatrix}
1 \\
1 \\
\vdots \\
1 \\
\vdots \\
1 \\
1
\end{bmatrix}
\qquad \text{equation 3}
$$

$$
\therefore D_a = D_b = 0.5
$$

This argument highlights a current problem in unifying the neutral and niche-assembled perspectives. As currently formulated, it is not clear whether environmental heterogeneity and the associated distributional biases should be incorporated into Hubbell's model at the level of the metacommunity, the set of species occurring within a biogeographic realm, or the local community (p. 313). This problem is exacerbated by the fact that environmental heterogeneity will occur on different scales for different environmental variables. Incorporating realistic levels of environmental heterogeneity into the UNT will be a challenge for its proponents.

Another possible justification for ecological equivalence is dispersal limitation. It has been demonstrated theoretically that strong dispersal limitation can decouple phenotypic differences in species performance across environmental gradients from recruitment (Hurtt and Pacala 1995). In other words, it doesn't matter if a species is well suited to a particular site if it never gets there. Although Hubbell et al. have provided evidence that the forest on Barro Colorado Island in Panama is strongly dispersal limited (1999), evidence from a mainland forest with an intact faunal assemblage indicates that the strength of dispersal limitation will differ greatly among forests (Webb and Peart 2001). Clearly this is an important topic for future research.

It is not within the scope of this comment to make any sort of overall judgment on the UNT and its utility to ecology. In fact, I agree with Hubbell that 'neutral' dynamics probably play a large role in structuring some ecological communities, including tropical forests. However, the invocation of fitness manifolds is insufficient to reconcile observed ecological differences among species with the assumption of ecological equivalence.

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Chapter Three: **Shifting patterns of biomass allocation in seedlings of the**

tropical dry forest tree *Gliricidia sepium* in response to soil quality:

a developmental reaction norm approach.

Abstract

The balanced growth hypothesis, or optimal allocation theory, predicts that plants allocate biomass so as to maximize their ability to collect the resource that is most limiting to their growth. In low nutrient soils, plants are expected to increase allocation to belowground foraging by increasing the amount of biomass allocated to the root system. The generality of this phenomenon is not known, however, and it has rarely been demonstrated for woody plants. In part, this is because many studies have not distinguished between changes in morphology that occur over the course of development (allometries) and those changes that are a direct response to the resource environment (adaptive plasticity). Also, many studies have used root:shoot ratio as a measure of allocation to belowground foraging, ignoring the fact that much root system biomass serves a structural role. Seedlings of the tropical dry forest tree *Gliricidia sepium* were grown in a common garden in three field collected soils from a Costa Rican conservation area. Two experimental designs were employed, one that accounted for allometry and one that did not. In the allometric design, plants were shown to be plastic in their biomass allocation patterns, and the observed plasticity was consistent with the balanced growth hypothesis. In the non-allometric design, plasticity was confounded with allometry and the results were much less informative. Comparisons between allocation to lateral roots

and taproots were made separately and combined as root:shoot ratio. Because of the greater mass of the taproot, patterns of allocation to lateral roots were obscured when root:shoot ratios were compared across soils.

Introduction

All things being equal, plants should allocate proportionally more energy towards collecting the resource most limiting to their growth (Bloom et al. 1985). As such, it is expected that plants will respond to differences in soil resource availability with increased allocation to roots, plasticity in the morphological and physiological characteristics of the root system such that the per gram capacity for resource uptake increases, or both (McConnaughay and Coleman 1999). The mechanisms by which plants perceive their light environment and respond to that information with morphological and physiological adjustments are well understood and appear to be quite general (Aphalo and Ballare 1995). In contrast, due to the relative difficulty of observing root system function *in situ* the occurrence and generality of plastic responses in the root system remains poorly documented. This is especially true for woody plants.

Two major methodological shortcomings contribute to the current confusion regarding the generality of plastic response in plant root systems. First, besides capturing resources, roots support the plant and serve as a site for the synthesis and storage of materials (Casper et al. 1998). Still, many studies have interpreted an increase in root:shoot ratio (total belowground biomass/ total aboveground biomass) as evidence that a species increases allocation to nutrient foraging functions in low nutrient conditions

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(e.g. Cahill 2003). Biomass allocated to a taproot or other anchoring structures is probably not involved in nutrient uptake, however. Because the surface area of the root system is a factor in determining nutrient uptake capacity of an individual, fine roots are much more important in nutrient uptake due to their much higher surface area: mass ratio. For the same reason, fine roots make up a very small proportion of root system biomass. Therefore, an increase in allocation to those roots actually involved in foraging for nutrients would be much more suggestive of an adaptive response to low nutrient conditions than an increase in belowground biomass.

Secondly, until recently most studies have failed to differentiate between developmental allometries and the putative morphological response of the plant to the environment (e.g. Bernston et al. 1995, Huante et al. 1995, Paz 2003). This typically occurs because allocation patterns are compared at a single point in time, and differences in overall growth rate between treatments are not considered. For example, a commonly observed growth allometry in plants is that root:shoot ratio decreases with plant size. In the case of Huante et al. (1995), seedlings of 34 species of tropical dry forest tree were grown for 60 days under two very different nutrient conditions. Plants in the high nutrient treatment were, on average, 9.5 times larger (by dry mass) than plants in the low nutrient treatment. Not surprisingly, all but three species exhibited a significantly higher root: shoot ratio in the low nutrient treatment. The difference was interpreted by the authors to mean that the plants had increased allocation to nutrient foraging functions in response to low nutrient conditions. Although this may have occurred, the authors could not have detected such a shift because their experimental design implicitly assumed isometry (the

special case of allometry in which the slope of the relationship between the two factors is one) between root biomass and shoot biomass. Although this problem has been pointed out repeatedly (Evans 1972, Gebauer et al. 1996, Gedroc et al. 1996, Casper et al. 1998, McConnaughay and Coleman 1999, Cahill 2003), published reports continue to make statements about adaptive morphological adjustment without accounting for this potentially confounding factor (e.g. Paz 2003) and if posters presented at ecological meetings are any indication, the trend is likely to continue into the future (pers. obs.).

When growth rate differs between treatments, in order to detect an adaptive shift in allocation patterns it is necessary to compare same-sized plants. Otherwise the difference being detected may simply be a difference in the rate of movement down a particular developmental trajectory, rather than a change in the trajectory itself (see figure 1 in McConnaughay and Coleman 1999). In practice, this can be achieved by collecting data in such a way as to be able to construct the developmental reaction norm (DRN) for a given set of treatments. The DRN for a species can be defined as the set of allometries obtained across an environmental gradient of interest (Schlicting and Pigliucci 1998). Statistical differences in the form of these allometries demonstrates phenotypic plasticity in the trait or traits being examined. The DRN is the ideal metric of comparison for allocation studies because it contains information on both developmental trajectories and the endpoints of those trajectories over a sampling interval. These endpoints will be important anytime growth is constrained to occur within a certain interval, or is continuous but periodical (as when it is interrupted by a period or periods of dormancy).

In order to construct the DRN, measurements must be made sequentially over the developmental interval of interest rather than only at the end of the interval.

The purpose of this experiment was to determine the extent to which whole-plant biomass allocation patterns in seedlings of the tropical dry forest tree *Gliricidia sepium* (Fabaceae: Papilionoideae) are affected by differences in soil quality. Of particular interest is whether or not *G. sepium* seedlings respond to low-nutrient soils by increasing allocation to the parts of the root system involved in foraging for nutrients, as predicted by the balanced growth hypothesis. The second goal of this study is to demonstrate the consequences of the shortcomings mentioned above for the interpretation of plant responses to the environment. This will be achieved by comparing results obtained using a DRN approach to results obtained without considering plant allometries and by comparing allocation patterns obtained when plant biomass is divided between leaf, stem, taproot and lateral root components to allocation patterns obtained from the root:shoot ratio.

If allocation patterns in *G. sepium* are plastic with respect to the availability of belowground resources significant differences between the growth allometries that make up the DRN across a range of soils that vary in nutrient availability is expected. Specifically, I predict that more biomass will be allocated to lateral roots in low quality soil, and more biomass will be allocated to leaves in high quality soil. If allocation patterns in *G. sepium* are not plastic with respect to soil, it is expected that the growth allometries that make up the DRN will be colinear at all sizes for which between-treatment comparisons can be made.

Methods

Study Site

 The study was conducted from July to November 2000 in Sector Santa Rosa of the Area de Conservación Guanacaste (ACG), a 110,000 hectare conserved wildland in northwestern Costa Rica. Sector Santa Rosa is characterized by remnant patches of dry deciduous and semi-deciduous forest of 0-400 years of age (Janzen 1986, 1988). Annual precipitation averages 1500 mm a year with most falling between late May and early December, with a two week break in August.

Species and soils

 G. sepium is very common as a small tree along roadsides and in regenerating pastures, although it is possible to find very large individuals in secondary and old growth forest, especially on rockier and more exposed sites. *G. sepium* has low survival and slow growth in deep shade (Klemens chapter 1) and seedlings and juvenile trees are commonly encountered in regenerating pastures. *G. sepium* is precocious with first reproduction occurring as early as two years. It is drought deciduous with explosion dispersed seeds.

 The three soils used in the experiment will be referred to as Pitilla (poor quality), Santa Rosa (intermediate), and Naranjo (high). Some chemical properties of these soils are presented in Table 1. Santa Rosa is probably an Inceptisol (USDA 1998) which formed as the volcanic material which forms the Santa Rosa plateau weathered in place. This soil lacks well-defined horizons. The textural class of this soil is clay loam. This soil was collected from young (20-30 yrs.) secondary successional forest near the

administration area of the ACG. Naranjo soil is from the low lying area between the Santa Rosa plateau and the Pacific Ocean. It is probably also an Inceptisol (USDA 1998) but is formed of a mixture of marine sediment and sediment which has washed down from the Santa Rosa plateau. The forest covering this area is also young (approximately 30 yrs.), but is characterized by a higher proportion of large trees than is the Santa Rosa soil, indicating that Naranjo soils may be particularly productive. Naranjo soil is also a clay loam and is texturally very similar to Santa Rosa soil. Pitilla soil is from the Atlantic slope of the Cordillera de Guanacaste, a volcanic formation which throws an orographic "rain shadow" over the Santa Rosa and Naranjo areas. Pitilla soil was collected from a site approximately 30 km NE of where Santa Rosa soil was collected. Pitilla soil thus receives 3000-4000 mm rainfall/year, while the Santa Rosa and Naranjo areas receive approximately 1500 mm rainfall/year. Texturally, Pitilla is a clay, is probably an Oxisol (USDA 1998)and is very red, which indicates that it is highly weathered and dominated by iron and aluminum oxide clays. The forest from which this soil was collected is young secondary succession with many thin tall trees and a dense understory of vines and shrubs.

Gliricidia is a tree of tropical dry forest. It does not occur on the Atlantic side of the volcanoes except where planted by humans. Thus it naturally occurs on the Santa Rosa and Naranjo soils, but does not naturally occur on the Pitilla soil.

Experimental Layout and Data Collection

Seeds of *G. sepium* were planted into black polyethylene nursery bags 25 cm deep and 15 cm diameter on 8 July 2000. Seeds were planted three to a bag and were thinned

to one plant per bag 10 days after planting. Bags were filled with one of the three soils described above. Bags were arranged evenly throughout a large light gap in secondary vegetation and were weeded throughout the experiment in order to prevent contamination of samples with other roots. Four plants were randomly selected and harvested approximately every two weeks for the duration of the growing season. The final harvest took place on 25 November 2000. Plants were harvested 18, 33, 47, 60, 74, 88, 102, 116, and 140 days after planting for a total of 36 plants harvested per treatment. Plants harvested on day 18 had not yet dropped their cotyledons. Because cotyledon biomass was interpreted as unallocated material, plants from the first harvesting date were not included in the analyses presented below leaving 32 plants per treatment.

At harvest, plants were clipped at the soil surface. Intact roots were cleaned of soil and the remaining fine roots were recovered from the soil bag by wet-sieving the soil. The height and number of leaves on each plant was recorded. Each plant was separated into leaves, stem, taproot, and lateral roots. All individuals of *G. sepium* had a well-defined monaxial taproot at all sizes and lateral roots were easily identified because they were always much finer than the taproot and always arose from the taproot. Samples were dried in a 68 C drying oven for at least one week. All portions were then weighed to 0.0001 g on an analytical balance. The percentage of total biomass contained in leaves, stem, taproot, and lateral roots was calculated and will be referred to as the leaf mass ratio (LMR), stem mass ratio (SMR), taproot mass ratio (TMR), and lateral root mass ratio (LRMR). Root:shoot ratio (R:S) was also calculated for all plants.

A group of 52 additional plants was harvested at the end of the experiment in order to compare plant performance in the three soils and in order to have sufficient sample size to make non-allometric comparisons of morphological variables. These plants were set aside at the beginning of the experiment and were arranged in four blocks spread throughout the light gap. These plants were grown in nursery bags of the same dimensions as above, and were planted on 29 June 2000, and harvested between 23 and 29 November 2000. Two plants were excluded from the analysis due to extensive herbivore damage, which was quantified weekly on a subjective scale. The same data were collected as for the sequentially harvested plants. Additionally, total leaf area for each of these plants was calculated by taking a digital photograph of the harvested leaves on a white background with a ruler. The images were converted to bitmap files and leaf area was calculated using Scion Image 1.62c (Rasband 2001).

Data analysis

To determine whether the growth rate of plants differed in the three soils, biomass, height, and leaf area of the large group of plants collected between 23 and 29 November were compared across soils using a two-way ANOVA with soil and block as main effects. All response variables were approximately normally distributed. Soil was designated a fixed effect and plot a random effect. Orthogonal contrasts were used to compare differences between soil treatments. The contrasts were structured as follows: Naranjo and Santa Rosa, the two soils where *Gliricidia* naturally occurs, were compared to Pitilla; Naranjo and Santa Rosa soil were then compared. The significance of each contrast was assessed with a student's t-test.

In order to compare the growth allometries among treatments, LMR, SMR, TMR, and LRMR values for the sequentially harvested plants were plotted against total plant biomass independently for each soil treatment. Following Niklas (1994) the resulting relationships were fit by an exponential function of the form:

$$Y_2 = \beta Y_1^{\alpha} \qquad \text{(eq.1)}$$

Where Y_1 is total biomass and Y_2 is the biomass ratio of interest. In order to compare these curves using ANCOVA, the equation was converted to the linear form:

$$\log Y_2 = \log \beta + \alpha \log Y_1 \qquad \text{(eq. 2)}$$

This is equivalent to log-transforming Y_1 and Y_2 and fitting a straight line through the resulting plot. These plots are presented in Figure 1.

Soil-specific allometries were compared with an analysis of covariance (ANCOVA) with the logarithim of total biomass as the covariate. Separate ANCOVAs were carried out with log transformed values of LMR, SMR, TMR, LRMR and R:S as the response variables. Soil was the main effect in each comparison, and was treated as a fixed effect. To test that the slopes of the regression of the response variable on the covariate (log total biomass) did not differ among treatments, an assumption of ANCOVA, ANCOVAs were first conducted with soil and total biomass as main effects and with the soil X total biomass interaction term included. A significant soil X total biomass interaction would indicate that the slopes of the allometries were significantly different, and intercepts cannot be compared in this case. In cases where the soil X total biomass interaction was not significant, the ANCOVA was rerun without the interaction term. When slopes do not differ, ANCOVA tests the differences between the intercepts

(log b in eq. 2) of the linearized regressions (Sokal and Rohlf 1995). Orthogonal contrasts were used to compare differences between soils. Planned contrasts were as above.

For non-allometric comparisons between allocation patterns, an ANOVA with soil and plot as main effects (as above) was performed on the large group of plants harvested at the end of the experiment, with LMR, SMR, TMR, LRMR and R:S ratio as the response variables.

Results

Overall growth

Plants grown in the different soils differed significantly in total biomass, height, and leaf area at the end of the experiment (Table 2). Plot did not have a significant effect on any of the size measurements, but there was a significant soil X plot interaction on plant height. Mean values for the different soils are presented in Table 3. All orthogonal contrasts between soils were significant, with Naranjo > Santa Rosa > Pitilla for all growth measures (Table 3). In terms of total biomass, plants grown in Santa Rosa and Naranjo soils were 207% and 391% larger, respectively, than plants grown in Pitilla soil. In all growth measures Pitilla was more different from Naranjo and Santa Rosa (as demonstrated by the relative magnitude of the sums of squares in the orthogonal contrasts) than Naranjo and Santa Rosa were from one other (Table 3).

Soil-specific allometries

Log-log plots of the soil-specific allometries of LMR, SMR, TMR, and LRMR are presented in Figure 1. There was no significant soil X total biomass interaction on

LMR, SMR, or TMR (Table 4). There was, however, a marginally significant interaction effect on LRMR. The interaction results because at small sizes LRMR in Pitilla soils is much higher than in Santa Rosa or Naranjo soils, but at larger sizes LRMR converges on the Santa Rosa and Naranjo values (Figure 1d). The slopes of LRMR in Santa Rosa and Naranjo soils did not differ, however (Table 4). For LRMR only plants grown in Santa Rosa and Naranjo soils were included in the ANCOVA.

Results of the ANCOVAs for LMR, SMR, TMR, and LRMR are presented in Table 5. The covariate was highly significant in all ANCOVAs, while soil had a highly significant effect on LMR, SMR and TMR.

Orthogonal contrasts between soils are presented in Table 6. All orthogonal contrasts for LMR, SMR, and TMR were significant. LMR was highest in Naranjo soil and lowest in Pitilla soil. SMR and TMR were highest in Pitilla soil and lowest in Naranjo soil. For the comparison of LRMR between Santa Rosa and Naranjo, there was a trend for plants growing in Santa Rosa soil to allocate more biomass to lateral roots, but it was not significant.

The log-log plot of R:S on total biomass is presented in Figure 2. The slope of the regressions did not differ among soils ($p > 0.25$), and both the main effect ($F = 26.00$; $p < 0.0001$) and the covariate ($F = 25.33$; $p < 0.0001$) terms were highly significant. R:S was highest in Pitilla soil and lowest in Naranjo soil. All orthogonal contrasts were significant and again Pitilla was more different from Santa Rosa and Naranjo than Santa Rosa and Naranjo were from one another.

When morphological characteristics were compared among the plants harvested simultaneously at the end of the experiment (Figure 3), a significant effect of soil was detected only for LRMR (F = 5.04; p < 0.0115). Orthogonal contrasts were significant and indicated that Pitilla was more different from Santa Rosa and Naranjo than Santa Rosa and Naranjo were from one another.

Discussion

Overall growth

Soil had a large effect on plant growth for all three measures of plant size. Since for tropical tree seedlings the probability of mortality is known to decrease with plant size throughout life (Lieberman 1996), seedling growth performance is probably a good fitness proxy in this system. As such, the soils included in this experiment represented a meaningful environmental gradient for this species. The significant soil X plot interaction for plant height was driven by the great variation in plot means for Naranjo soil. Since there was no significant interaction term for total biomass, it is probable that the quality of the light reaching the plots differed more than the amount, and the variability in height was a slight etiolation response that was only detectable in plants grown in Naranjo soil due to their larger overall size.

Patterns of biomass allocation

Soil strongly affected patterns of biomass allocation in both aboveground and belowground plant parts. The slope of the allometries for LMR, SMR, and TMR did not differ with respect to soil, but the significant differences in the intercept indicated that

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plants differed in the fraction of biomass allocated to leaves, stems, and taproot over the growth interval observed.

The lack of homogeneity between slopes indicates that there was a difference in the form of the growth allometry for biomass allocated to lateral roots. Plants grown in Pitilla, the lowest quality soil, initially invested more biomass in roots than did plants in the higher quality soils, although they eventually converged on Naranjo and Santa Rosa values (Figure 1d).

Overall, these results are consistent with the balanced growth hypothesis. The tree seedlings studied shifted their developmental pathways to allocate proportionally more of their biomass towards leaf material as soil quality increased and at least initially allocated more biomass towards root material in the lowest quality soil. Although these results are consistent with balanced growth, for an individual plant any biomass allocated to one tissue can't be allocated to another so it is difficult to say what factor is *driving* the observed patterns and which changes are secondary consequences.

In all soils, the proportion of biomass allocated to leaves and lateral roots decreased with plant size, while the proportion allocated to stem and tap root tissue increased with plant size. As the plant gets taller and acquires more leaves the stem must get thicker and more highly branched in order to get the same amount of separation between leaves. Increases in allocation to the taproot may represent an increased need for belowground support as the stem grows taller and heavier. Because dry forest seedlings need to survive a six month dry season after six months of growth, another explanation may be that the taproot may be storing carbon reserves, or is programmed to get as deep

as possible before the rains stop in order to maintain access to soil water for as long as possible. In other words, the marginal costs of nutrient foraging, in this case expressed as architectural or ecological constraints, are increasing faster than the rate of return per unit of leaves and roots (Bloom et al. 1985).

Unlike the other allometries, there was no significant difference between the Naranjo and Santa Rosa LRMR allometries. Lumping all lateral root material into the same category may have reduced the likelihood of detecting differences. Because fine and thick lateral roots were not separated in this experiment (secondarily thickened lateral roots began to develop quite early on, and large plants at the end of the experiment had quite thick lateral roots), the increase in architecturally necessary thick laterals that are not directly involved in uptake (another marginal cost) increase the mass of the lateral root system disproportionate to their effect on nutrient uptake. In order to separate out this effect we would need to know the relationship between root system biomass and root system surface area. Although separating root material in this way may be an improvement over using root: shoot ratio (see below) it is still a very crude measure of belowground morphology.

Root:shoot ratio and leaving out allometry

Because of the large absolute differences between biomass allocated to lateral roots and the tap root (Figure 3), root: shoot ratio was driven mostly by taproot biomass. As a result, in contrast to LRMR, root: shoot ratio increased over the course of development. The ANCOVA of root:shoot ratio showed no interaction between total biomass and soil, and there were clear differences in the intercepts of the three

allometries. Had root: shoot ratio been used as a proxy for allocation to below- versus above-ground acquisition, the results still would have supported the balanced growth hypothesis, but because LRMR was swamped by TMR, the result would have been mostly driven by the increased allocation to leaves in higher quality soil. This effect is undoubtedly of less importance in plants with fibrous root systems, but probably needs to be taken into account whenever the taproot accounts for a disproportionate amount of the belowground biomass.

If plants had been harvested only at the end of the growing period the relevant shifts in allocation patterns would have been completely obscured (Figure 3). The pattern of results are almost completely reversed from the allometric comparison. There is no significant effect of soil on LMR, SMR, TMR or R:S, and a significant effect of soil on LRMR, the one response variable that showed the least separation between allometries in the allometric analysis.

The message here is clear. Studies which claim to demonstrate adaptive plasticity (or the lack thereof) in allocation patterns in response to environmental variation must consider developmental allometries if experimental treatments cause overall differences in plant size.

How adaptive was the plasticity?

The major problem in demonstrating that a particular plastic response is adaptive is that the relationship between the response of a trait to an environmental gradient and the fitness response of an organism may take any form. Plasticity may be adaptive and yet fitness may stay the same, increase, or decrease. Therefore, the only way to show

whether or not plasticity is adaptive is to demonstrate that the plant would have lower fitness were the trait not plastic (Schlicting and Pigliucci 1998). This can be achieved by inducing an organism to develop the 'wrong' phenotype for a particular environment and comparing its resulting fitness to an organism showing the 'correct' phenotype (e.g. Schmitt et al. 1995). However, for a phenotype such as root system morphology, or a developmental phenotype such as the pattern of biomass allocation, inducing the wrong phenotype may be impossible.

The one thing that is obvious is that although there was considerable plasticity in the response to soil, and the response was consistent with an expected adaptive response to environmental conditions, whatever fitness benefits accrued from that shift were not nearly enough to equal out the performance differences across environments.

Past studies

Several other studies have demonstrated shifts in biomass allocation patterns in response to soil while taking allometry into account (Gebauer et al. 1996, Gedroc et al. 1996, McConnaughay and Coleman 1999, Shipley and Meziane 2002). That the observed differences occurred relatively early in the developmental process is consistent with the results of McConnaughay and Coleman (1999) for roots and with Bonser and Aarssen (2003) for aboveground parts. Shipley and Meziane (2002) found evidence for optimal allocation in response to variation in the nutrient environment to be general in a study of 22 species of herbaceous plants, while Müller et al. found little evidence for it in a study of 27 (2000). Muller's harvests were spread over a much longer time period and had much lower replication per species. If shifts in biomass allocation generally take place

earlier in development rather than later, it is quite possible that the wider sampling intervals of Müller et al. reduced statistical power of their analyses to below the point where shifts in allocation could be detected. Casper et al. (1998) and Cahill (2003) did not detect changes in allocation responses when plants were exposed to different levels of above- and belowground competition, respectively.

In one of the only studies to look at allocation patterns in a woody plant and one of the few to consider biomass partitioning within different components of the roots system, Gebauer et al. (1996) showed that potted Loblolly pine seedlings increased both the amount of biomass allocated to lateral roots and the specific root length (SRL) (m root/ g root) of lateral roots when grown under low nitrogen availability and that this response was independent of ontogeny. Partitioning the root system into different components may seem to be more important in woody plants than in herbaceous species, but in herbaceous species with well developed taproots, the response of lateral roots may still be swamped by taproot biomass (Casper et al. 1998). Furthermore, many studies have demonstrated that plants are very plastic within the root system because they are able to proliferate roots in areas of high nutrient concentration (see refs in Casper and Jackson 1997) although this has not yet been related back to patterns of whole-plant biomass allocation.

Obviously, a clear consensus on the generality of balanced growth has yet to emerge.

Statistical problems stemming from the use of ratios

Some will undoubtedly argue that the relationships reported in this study are meaningless due to spurious correlations that arise in comparisons of parts and wholes of organisms (Cahill 2003). In reality, this critique is a result of the application of inappropriate null models. In a much cited paper, Jackson and Somers (1991) show that although two variables, X and Y, are randomly distributed with respect to one another, the resulting plot of Y/X on X (the case of the part-whole correlation) shows a negative slope. From this, they argue that the appropriate null hypothesis for a part-whole correlation is not a correlation coefficient of zero. They argue that to detect a significant relationship between a part and a whole it is necessary to randomize Y on X and to compare the observed correlation coefficient to the observed 'null' distribution of correlation coefficients. This leads to the counterintuitive result that an observed r of zero between a part and a whole may be significant, while an observed r of –0.7 may not.

This result is counterintuitive because it has no biological meaning. Randomizing Y on X and recalculating the ratio at each step removes from consideration the initial size dependence for which the use of the ratio was meant to account. Allocation studies assume a relationship between the part and the whole. The question of interest is whether or not that relationship is dependent on the total size of the organism. The appropriate null distribution is therefore obtained by randomizing the set of observed relationships, Y/X, on X. Performing this randomization gives the null correlation of zero. This is an appropriate null hypothesis, as it represents the biologically meaningful case of isometry. Ruf (2000) has recently made a very similar argument in a different biological context.

Implications at the community level

Determining the generality of balanced-growth or optimal allocation type responses among plants is important because the capacity for plastic response will play a role in determining the fitness that results from any genotype X environment combination in nature. If plasticity were uniformly strong and unconstrained, for example, it would serve to make species functionally ecologically equivalent (*sensu* Hubbell 2001). Exploring species' plastic responses to those variables that we suspect are ecologically operative will therefore play a fundamental role in determining the role of species traits in structuring biological communities.

Tables

Table 1. Some chemical and physical properties of the soils used. O.M. % is the percentage organic material by weight in the sample, determined by the Walkley-Black acid digestion method. CEC is the cation exchange capacity of the sample, and was determined directly. pH was determined in water. Phosphorous and Potassium were determined by a modified Olsen method. Ca, was extracted with 2M KCl. All values below are averages (n=2) of two samples taken in August 2000.

soil	O.M. %	CEC	pH	P (mg/Kg)	Ca (cmol(+)/Kg)	K (cmol(+)/Kg)
Naranjo	2.4	30.6	7.3	15.25	20.1	0.76
Santa Rosa	4.8	25.7	6.1	11.1	13.0	0.66
Pitilla	3.9	17.7	4.9	11.9	3.2	0.075

Table 2. ANOVA results for Biomass, Height, and Leaf area at the end of the experiment. p values < 0.05 are in **bold**.

Factor	df	Biomass (g)			Height (cm)			Leaf Area (cm^2)		
		SS	F	p	SS	F	p	SS	F	p
Soil	2	1562	32.7	**0.0003**	4198	9.92	**0.0108**	950009	11.2	**0.0072**
Block	3	254	3.52	0.0826	784	1.21	0.379	1083255	0.842	0.516
Soil X Block	6	147	1.41	0.236	1337	2.55	**0.0356**	2622938	1.50	0.206
Error	38	658			3318			11100929		

Table 3. Number of plants and mean value (± Standard Error) of biomass, height, and leaf area for plants grown in the three soils. Contrasts are the results of orthogonal contrasts between treatments, as described in the text. N = Naranjo, SR = Santa Rosa, P = Pitilla. (n) is number of plants. p values < 0.05 are in **bold**.

| | Means ± SE | | | Contrasts | | | |
| | | | | N, SR vs. P | | N vs. SR | |
Variable	N	SR	P	SS	p	SS	p
n	13	18	19				
Biomass (g)	21.3 ± 1.63	11.3 ± 1.16	5.45 ± 1.14	1256	**0.0003**	614	**0.0023**
Height (cm)	56.5 ± 4.86	43.7 ± 3.45	33.3 ± 3.39	3482	**0.0067**	1517	**0.0367**
Leaf Area (cm^2)	1645 ± 218	1003 ± 155	580 ± 152	$7.28*10^6$	**0.0061**	$4.17*10^6$	**0.0203**

Table 4. Results of the test for homogeneity of slopes for the ANCOVA with soil as the main effect and total biomass as the covariate, with the soil X log total biomass interaction included in the model. p is the p value for the soil X log total biomass interaction. LRMR* is the p value for the soil X log total biomass interaction after Pitilla values were removed from the analysis.

Variable	p
log LMR	0.650
log SMR	0.767
log TMR	0.307
log LRMR	**0.0506**
log LRMR*	0.340
log R:S	0.285

Table 5. Results of the ANCOVAs with soil as the main effect and log total biomass as the covaviate. Response variable LRMR* was calculated without the values for Pitilla (see text).

Factor	df	log LMR SS	F	p	log SMR SS	F	p	log TMR SS	F	p	df	log LRMR* SS	F	p
Soil	2	0.392	26.5	**<0.0001**	0.124	13.0	**<0.0001**	0.693	25.4	**<0.0001**	1	0.0350	3.24	0.0769
log total biomass	1	0.428	58.1	**<0.0001**	0.271	56.9	**<0.0001**	0.983	72.0	**<0.0001**	1	0.210	19.4	**<0.0001**
Error	95	0.679			0.439			1.26			61	0.660		

Table 6. Least squares means, back transformed, and 95% confidence intervals. Contrasts are the results of orthogonal contrasts between treatments, as described in the text. N = Naranjo, SR = Santa Rosa, P = Pitilla. p values < 0.05 are in **bold**. LRMR* did not include Pitilla plants.

| Variable | Least Squares Means, (95% CI) | | | Contrasts | | | |
| | | | | N, SR vs. P | | N vs. SR | |
	N	SR	P	SS	p	SS	p
LMR	0.499	0.407	0.329	0.322	**$2.6*10^{-9}$**	0.115	**0.0002**
	(0.463-0.538)	(0.380-0.436)	(0.305-0.353)				
SMR	0.276	0.320	0.348	0.0806	**$8.6*10^{-5}$**	0.0597	**0.0006**
	(0.260-0.293)	(0.303-0.338)	(0.328-0.370)				
TMR	0.121	0.158	0.210	0.574	**$4.3*10^{-9}$**	0.198	**0.0003**
	(0.109-0.134)	(0.144-0.173)	(0.190-0.233)				
LRMR*	0.0742	0.0831	--	--	--	--	--
	(0.0681-0.0808)	(0.0763-0.0906					

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Figures
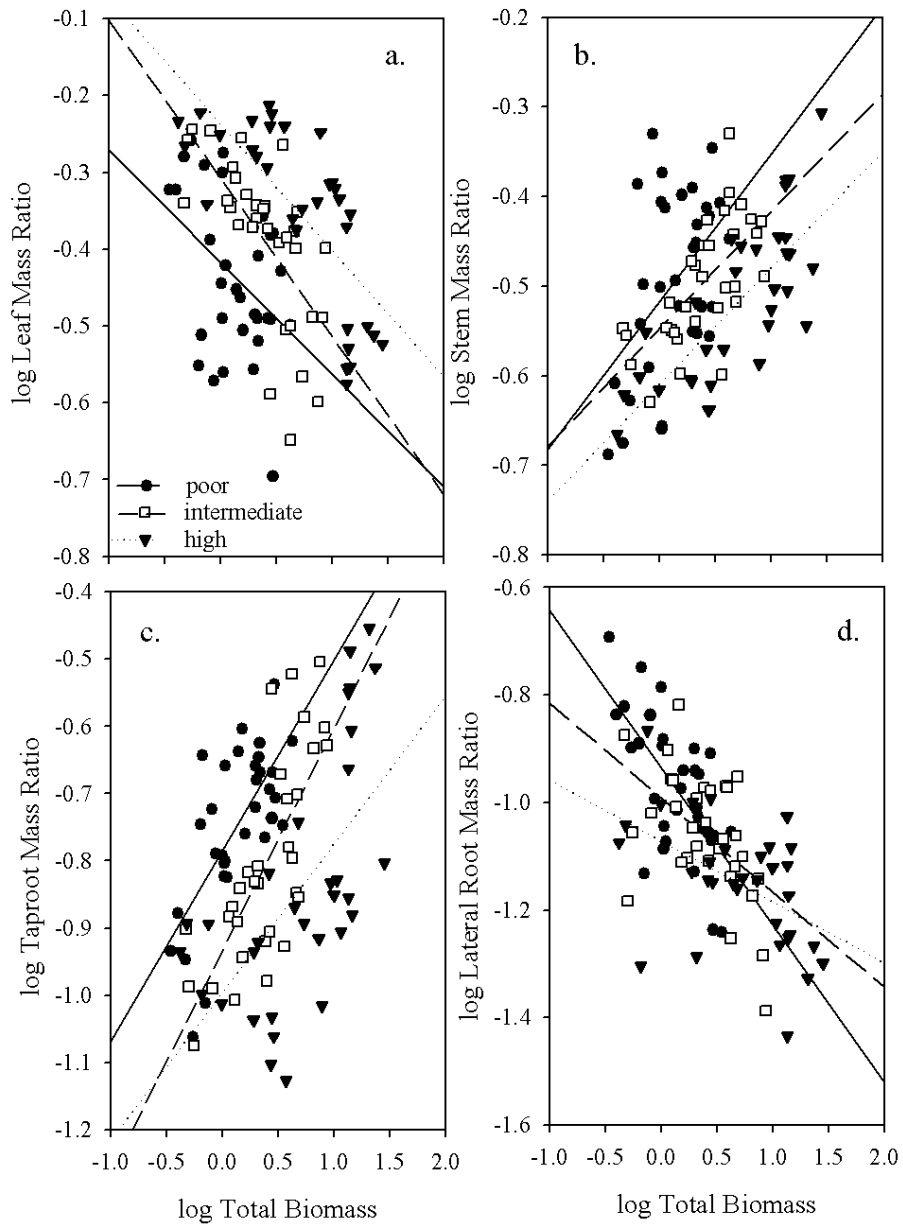
Figure 1. Log-log scatter plots with least squares regression lines of a) leaf, b) stem, c) taproot, and d) lateral root biomass ratios on plant size. Symbols represent poor (Pitilla), intermediate (Santa Rosa), and high (Naranjo) quality soils.

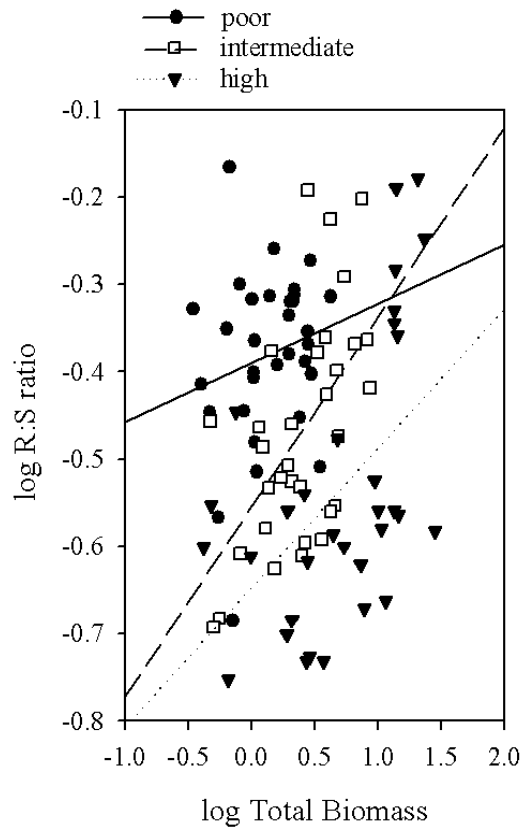**Figure 2. Log-log scatter plots with least squares regression line of root: shoot ratio on plant size. Symbols represent poor (Pitilla), intermediate (Santa Rosa), and high (Naranjo) quality soils.**
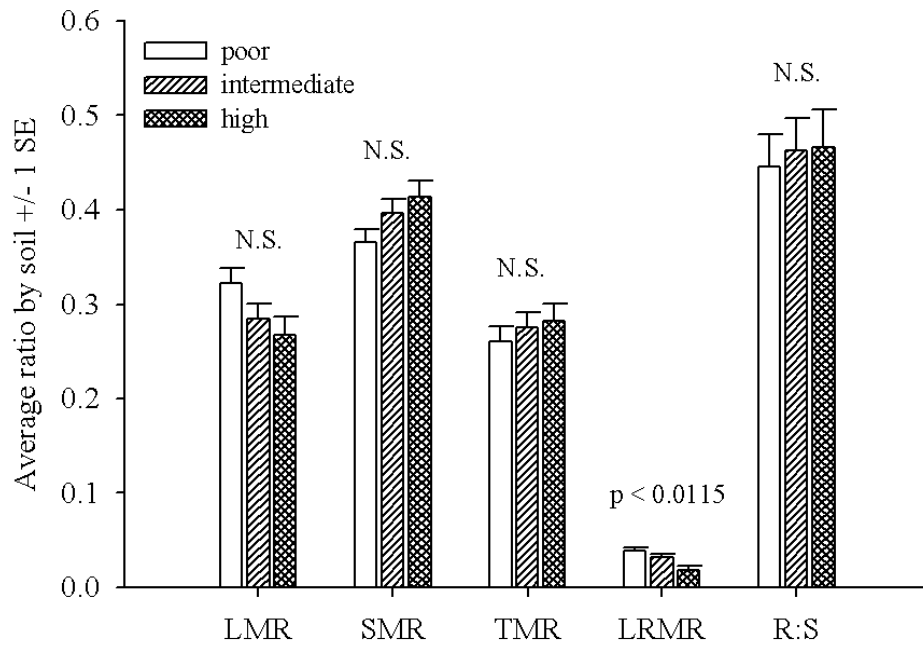
Figure 3. Organ biomass ratios and root: shoot ratio for plants harvested at the end of the experiment. N.S. = no significant difference. Error bars are ± 1 S.E.

Chapter Four: **Changes in biomass allocation and above- and belowground**

morphology in response to soil and light in tropical dry forest tree seedlings.

Abstract

Seedlings of two species of the tropical dry forest trees *Gliricidia sepium* and

Hymenaea coubaril were grown across all combinations of three light and three soil

treatments in the field in order to investigate changes in biomass allocation patterns and

morphology. These gradients were known to cause differences in the growth rates of

these species. Plants were harvested sequentially so that plant allometry could be

accounted for in the analysis. Despite differences in morphology, life history, and

responsiveness to the treatments, the two species showed strikingly similar patterns of

morphological change in response to the gradients. The effect of soil and light on lateral

root length, lateral root surface area, etiolation, specific leaf area, the ratio of biomass in

lateral roots to that in leaves, and the ratio of lateral root surface area to leaf surface area

were compared across treatments. In general, soil type had little effect on either above- or

belowground morphology or plant allocation patterns, while light had a consistent effect

that was typically manifested as a change in the intercept but not the slope of the log-

transformed allometry for the trait in question. The magnitudes of the plastic responses

were greater for *G. sepium*, the species which exhibits the larger growth response to the

light and soil gradients.

Introduction

For plants, developmental plasticity is an important buffer against environmental

heterogeneity. While the response of plant shoots and leaves to variation in the light environment has been well documented (Aphalo and Ballare 1995), little is known about belowground or whole-plant manifestations of adaptive plasticity. The root systems of woody plants have been particularly neglected.

Tropical dry forest (TDF) tree seedlings inhabit a challenging and extremely heterogeneous environment. Light availability can change dramatically at the meter scale, and landscape features such as topographic position or soil type will change at a scale of anywhere from tens to thousands of meters (Clark et al. 1998). In chapter 1, I demonstrated that soil can have a large effect on growth rates in TDF seedlings, but that species vary widely in their responsiveness to changes in the soil environment. When considering species' responses to the environment, it is important to consider the interactions between environmental variables that occur on these different scales.

In chapter 3, I documented a case of a tree species, *Gliricidia sepium*, adjusting its biomass allocation patterns in response to changes in the soil environment. The shifts in allocation, an increase in leaf material in high nutrient soils and an increase in lateral root material in low nutrient soils, were consistent with the balanced growth hypothesis, which suggests that a species should allocate biomass preferentially towards whatever resource is most limiting its growth (Bloom et al. 1985, McConnaughay and Coleman 1999). To understand the role of adaptive plasticity in root and shoot growth in nature it is important to understand how general is the phenomenon of plastic biomass allocation. Studies of adaptive plasticity that consider only a single environmental variable cannot make very strong claims for the generality of their results.

This paper presents a comparative study of whole-plant allocation patterns for two

species of tropical dry forest tree seedlings grown across gradients of light availability and soil quality. The species differ greatly in life history characteristics: one, *Hymenaea coubaril*, is relatively insensitive to changes in the light and soil environment while the other, *G. sepium*, showed the greatest growth response to both light and soil in the experiment presented in chapter 1. I consider examples of three kinds of plasticity: plasticity in aboveground morphology, belowground morphology, and whole-plant allocation patterns.

As I pointed out in chapter 3, when studying plasticity that is putatively adaptive, it is important to choose measurements that reflect allocation to the functions of interest as much as possible. This study is unique in that it includes detailed measurements of above- and belowground morphology in an attempt to make correct inferences about the role of the observed plasticity. Rather than employing crude measures such as root:shoot ratio, this study considers morphological measures that can be more directly related to functionality, such as etiolation, the surface area of the root system, and allocation to lateral roots and leaves.

Methods

The study was conducted from June to November 2001 in Sector Santa Rosa of the Area de Conservación Guanacaste (ACG), a 110,000 hectare conserved wildland in northwestern Costa Rica. Sector Santa Rosa is characterized by remnant patches of deciduous and semi-deciduous forest of 0-400 years of age (Janzen 1986). Annual precipitation averages 1500 mm a year with most falling between late May and early December, with a two week break in August. The three clay loam soils used in this

experiment have been described in chapter 1, table 1, and will be referred to as poor, intermediate, and high quality soils.

The two species are very different ecologically. *G. sepium* is very common as a small tree along roadsides and in regenerating pastures, although it is possible to find very large individuals in secondary and old growth forest, especially on rockier and more exposed sites. *G. sepium* has low survival and slow growth in deep shade (chapter 1) and seedlings and juvenile trees are commonly encountered in regenerating pastures. *G. sepium* is precocious with first reproduction occurring as early as two years. It is drought deciduous with explosion-dispersed seeds. *H. coubaril* is a slow growing, shade tolerant, evergreen, animal-dispersed species that is the most common component of certain types of old growth forests of upland Santa Rosa. *H. coubaril* mast fruits at long intervals and attains 400+ years of age. It occurs at low densities in a wide variety of forests in Central America and the Caribbean.

Plants were grown in the same 15 sites and three soils described in chapter 1. Each site was one of five replicates of three light treatments, shade, gap, or open. Instead of harvesting all plants at the end of the experiment, however, one randomly selected plant from each soil X species combination at each of the five replicate sites was chosen for harvest every two weeks for eight weeks, beginning three weeks after planting. In this way 360 plants of each species were harvested over the course of the experiment. Plants which had suffered greater than 10% leaf tissue loss to herbivores at any point during the experiment were excluded and never harvested. The first harvest of *H. coubaril* was also excluded from analysis because many individuals did not emerge until two weeks after planting and the largest biomass component at the time of harvest was undifferentiated

cotyledon material. Most *G. sepium* emerged within three days of planting and had dropped their cotyledons by the time of the first harvest.

Santa Rosa was extremely dry in 2001 (67 mm rainfall between 10 June and 31 July, compared to 202 ± 111 mm (average $\pm$ 1 SD) during the same period for all other years from 1994 to 2002), so plants were watered from shortly after planting until early August as described in Chapter 1. Watering was insufficient to break down the naturally occurring covariation between solar radiation and soil moisture due to the drought. It was obvious that soil bags in shade treatments retained more moisture than soil bags in gap treatments, and that soil bags in gap treatments retained more moisture than soil bags in full sun.

At harvest, each plant was clipped at the soil surface and the soil-bound roots were immersed in a bucket of water for one or two hours before cleaning. At the time of cleaning, the root system was extracted intact from the soil bag and the soil was wet-sieved in order to collect roots which broke off in the washing process. The vast majority of roots belonging to each individual were recovered.

In addition to the amount of dry mass in stems, leaves, taproot and lateral roots, a number of other morphological data were collected. The total leaf surface area (one-sided) for each individual was calculated by taking digital photographs of pressed leaves on a ruled background, and measuring the area of the leaves in the resulting images using the Scion Image program (Rasband 2001). The average specific leaf area (SLA) for each plant was calculated as the ratio of the dry mass of all leaves to leaf surface area. The etiolation response was measured as the ratio of stem diameter at 5 cm above the soil surface to plant height.

Measurements of the root system were made with the proprietary system WinRhizo version 2002c (Regent Instruments, Quebec City). This program measures root lengths and diameters directly by counting pixels on the scanned image. By assuming that roots are cylindrical, WinRhizo is also able to calculate the volume and surface area of the root system. In order to improve the accuracy of the measurements, lateral roots were separated from the taproot prior to analysis, and in the case of *G. sepium*, all nodules were removed from the root system before scanning and were weighed apart from the rest of the root system.

As any of the response variables could exhibit non-isometric allometry, all between treatment comparisons were made with analysis of covariance (ANCOVA) (Sokal and Rohlf 1995) as described in chapter 3. Because this study considered two environmental gradients simultaneously, ANCOVAs testing for the effect of soil were conducted separately within each of the light treatments, and ANCOVAs testing for the effect of light were conducted separately within each of the soil treatments. All variables were log transformed in order to linearize the allometries.

Six response variables were chosen to test the effect of light and soil on plant allocation. To test for plasticity in aboveground responses, etiolation and specific leaf area were compared. The etiolation response is expected to increase in low light and specific leaf area is expected to be higher for shade leaves, which must last longer in order to pay back their production cost (Reich et al. 1999). To test for plasticity in belowground responses, the total length and total surface area of the lateral roots were compared. For those four measures, total plant biomass was employed as the covariate in the ANCOVA. To test for shifts in whole plant allocation patterns, the allometry of

lateral root mass on leaf mass, and the allometry of lateral root surface area on leaf surface area were compared. The balanced growth hypothesis predicts that plants should allocate proportionally more resources to the resource most limiting to growth (Bloom et al. 1985). If this is true for these species, we would expect to see increased lateral root biomass, length, and surface area in lower quality soils, and increased allocation to leaves and lower allocation to roots in low light.

Results

Growth rate and structure

As demonstrated in Chapter 1, *G. sepium* and *H. coubaril* differed in their growth response to the light and soil conditions they encountered in this experiment (Figure 1). *H. coubaril* is relatively insensitive to differences in soil type and shows much less response to the light gradient than does *G. sepium*.

Both species' root systems were dominated by a well-defined taproot with lateral roots arising axially around it (

Figure **2).** Lateral roots of *H. coubaril* were woodier and more brittle than those of *G. sepium*. Several orders of branching could be observed on *G. sepium* roots while *H. coubaril* rarely branched beyond the third order and secondary and tertiary roots were never long. In contrast, *G. sepium* had long fine roots that branched often and penetrated throughout the soil volume contained in the bag. Also, large individuals of *G. sepium* developed a thick, carrot-like taproot, while the taproot of *H. coubaril* remained relatively thin as the root system developed.

Surprisingly, considering the structural differences, the effective size of the root system, measured as the allometry of surface area on plant size, did not differ greatly

between the two species when they were compared over all light and soil treatments (Table 1, Figure 3). Differences in the intercept of the allometry indicate that Gliricidia has a larger root system surface area per unit of plant mass at all sizes, but the slope of the allometry is nearly identical. There was no discernible difference between the two species in either the slope or the intercept in the lateral root surface area/ leaf surface allometry (Table 1, Figure 3).

Belowground morphology

Belowground morphology was much more affected by light than by soil. All 12 comparisons of the effect of light on lateral root length and lateral root surface area reveal either highly significant effects of light or highly significant interaction terms, which reveal that the form of the allometry changes across environments (Table 2, Figure 4). In all of these cases, the allometry was biased upwards in the high light environment for part or all of the developmental trajectory (Figure 4), indicating that lateral roots were produced in response to higher light, as expected. It is possible that the interactions in the allometries, particularly in the case of *G. sepium* arose not due to actual difference between the slope of the allometry but rather as a result of insufficient overlap between the sizes of the plants in the shade environment and plants in the high light environment at the time of harvest. Soil affected root morphology only in shade-grown *H. coubaril* and open-grown Gliricidia (Table 2). In both cases, however, the most roots were produced in the high quality soil and the least in the low quality soil, contrary to prediction.

Aboveground morphology

Aboveground morphology was also more strongly affected by light than by soil.

There was no consistent pattern of response in either SLA or etiolation to soil (Table 3, Figure 5). Only one main effect was significant, *H. coubaril* had slightly denser leaves in the poorest soil. Not surprisingly, the light environment had a large effect on both etiolation and SLA. As predicted, the etiolation response was strongest in the shade for both species, although the magnitude of the response was consistently much larger for *G. sepium* (Figure 5). Counter to prediction, SLA was consistently highest (leaves were densest) in the high light environment for both species. Also of note is the fact that the allometries were generally much weaker (they explained a much smaller proportion of the variation relative to the main effect) for these aboveground traits than for any of the other traits studied.

Balanced growth

In the comparisons of root to leaf allocation, soil type caused shifts in only two cases, both of the measures of allocation for *G. sepium* in open light (Table 4, Figure 6). The shifts were small in magnitude and in the opposite direction of the predictions of balanced growth, with the highest allocation to roots in the highest quality soil. In contrast, light had a highly significant effect on both measures of allocation in both species across all three soils (Table 4, Figure 7). The response to the light gradient was in agreement with the predictions of balanced growth, with higher allocation to roots in the high light environment (Figure 7).

Discussion

Despite the large growth response of *G. sepium* in the high quality soil, the biomass allocation patterns of both species in this study were insensitive to the soil gradient. In contrast, all of the morphological variables measured in both species were sensitive to changes in the light environment regardless of soil.

The light and soil gradients in this experiment were not independent, however, due to the drought. As mentioned above, shaded sites retained moisture better than sites in the open treatment even with supplemental watering. The high quality soil was slightly sandier and much more friable than the low or intermediate soils, so there may have been a gradient of water availability across the sites and the slight increase in *G. sepium* root allocation in high quality soil may be a reflection of that difference. Likewise, SLA values may have been higher in open sites due to the plant producing more xerophytic leaves under drought conditions.

The magnitude of the plastic response in *G. sepium* was usually greater than that observed in *H. coubaril*, which is consistent with *G. sepium*'s ability to respond to favorable conditions with extremely rapid growth. However, comparisons of the amount of plasticity between species must be made with great care. The reality of any measured amount of plasticity, whether the slope of a reaction norm or the difference in intercepts between two regression lines in a developmental reaction norm, is constrained by the range of environments included in the study in question . Rather than making the assumption that the range of environments chosen for a particular study is equally ecologically relevant to all species, it needs to be asked how well that range of environments agrees with the range of environments a species would encounter in nature. In this case, *G. sepium* was challenged with the entire range of light environments in which it can survive, while *H. coubaril* seedlings can persist in considerably deeper shade than what they experienced in this experiment.

The lack of a balanced growth adjustment in response to soil in *G. sepium* seems to contradict the findings of chapter 3. In the earlier experiment the shift in lateral root mass ratio was observed only in a soil in which *G. sepium* does not naturally occur. In contrast, *G. sepium* naturally occurs on all soils used in this experiment. The gradient in the last experiment may have been unrealistically steep. On the other hand, it may simply be that the uncontrolled variable swamped out any effect of nutrient availability

on development. Regardless, this experiment highlights the difficulty of building general

models of plant growth in natural environments.

Tables

Table 1. Comparison of growth allometries across all treatment levels between

G. sepium and *H. coubaril*

	G. sepium			*H. coubaril*		
	r^2	slope	intercept	r^2	slope	intercept
log root surface area X log total mass	0.88	0.953	1.799	0.87	1.089	1.554
log lateral surface area X log leaf area	0.62	1.268	-1.219	0.54	1.251	-1.036

Table 2. ANCOVAS of effect of soil and light on belowground morphology. Significant main effect and interaction p values in **bold**. NS = not significant.

	Lateral root length (cm)						Lateral root surface area (cm²)					
	G. sepium			*H. coubaril*			*G. sepium*			*H. coubaril*		
	SS	F	p	SS	F	p	SS	F	p	SS	F	p
soil(shade)	0.202	0.7466	0.48	0.249	4.483	**0.015**	0.258	0.6239	0.5379	0.193	3.316	**0.042**
total mass	8.66	64.05	<0.0001	1.03	36.95	<0.0001	9.25	44.70	<0.0001	1.18	40.51	<0.0001
interaction	N.S.			0.267	4.806	**0.011**	N.S.			0.308	5.272	**0.0074**
soil(gap)	0.131	2.632	0.076	0.0189	0.7677	0.47	0.149	2.834	0.063	0.0220	0.7603	0.47
total mass	5.71	230.1	<0.001	1.70	137.7	<0.0001	7.25	275.2	<0.0001	2.83	195.2	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		
soil(open)	1.08	12.26	**<0.0001**	0.0138	0.3923	0.68	1.36	28.56	**<0.0001**	0.0592	1.383	0.26
total mass	2.11	47.83	<0.0001	2.78	157.5	<0.0001	2.52	106.3	<0.0001	4.85	226.7	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		
light(poor)	1.799	20.45	**<0.0001**	0.398	12.80	**<0.0001**	6.27	60.59	**<0.0001**	0.478	13.00	**<0.0001**
total mass	3.740	85.06	<0.0001	1.71	110.3	<0.0001	3.66	70.72	<0.0001	2.92	158.9	<0.0001
interaction	0.375	4.262	**0.0165**	N.S.			N.S.			N.S.		
light(intermediate)	1.39	8.756	**0.0003**	0.816	18.89	**<0.0001**	1.69	7.090	**0.0013**	0.235	4.680	**0.012**
total mass	5.11	64.17	<0.0001	1.01	46.90	<0.0001	6.89	57.91	<0.0001	1.38	54.84	<0.0001
interaction	0.973	6.104	**0.0030**	N.S.			1.33	5.573	**0.0049**	0.190	3.795	**0.027**
light(high)	0.808	5.520	**0.0053**	0.269	6.493	**0.0026**	0.964	6.981	**0.0014**	8284	6.812	**0.0020**
total mass	7.17	98.01	<0.0001	2.85	137.7	<0.0001	8.38	121.4	<0.0001	9890	161.3	<0.0001
interaction	1.46	9.958	**0.0001**	N.S.		**0.0001**	1.66	12.06	**<0.0001**	26610	21.89	**<0.0001**

Table 3. ANCOVAS of effect of soil and light on aboveground morphology. Significant main effect and interaction p values in **bold**. NS = not significant.

| | Etiolation (height/diameter) | | | | | | SLA (g leaf/ cm^2 leaf) | | | | | |
| | *G. sepium* | | | *H. coubaril* | | | *G. sepium* | | | *H. coubaril* | | |
	SS	F	p	SS	F	p	SS	F	p	SS	F	p
soil(shade)	0.00464	0.2021	0.82	0.0535	1.537	0.22	0.0065	0.3355	0.72	0.00145	0.213	0.81
total mass	0.137	11.99	0.0008	0.128	7.347	0.0084	0.146	15.02	0.0002	0.0254	7.48	0.0079
interaction	N.S.			N.S.			N.S.			N.S.		
soil(gap)	0.0120	0.7667	0.47	0.00295	0.3168	0.73	0.0272	2.318	0.10	0.00782	1.86	0.16
total mass	0.0000452	0.0058	0.94	0.0352	7.5379	0.0076	0.360	61.20	<0.0001	0.00134	0.639	0.43
interaction	0.0651	4.1662	**0.0180**	N.S.			N.S.			N.S.		
soil(open)	0.00760	0.4723	0.62	0.0141	0.3817	0.68	0.000686	0.0856	0.92	0.0255	4.296	**0.017**
total mass	0.241	29.97	<0.0001	0.0349	1.885	0.17	0.0458	11.46	0.0010	0.0178	5.974	0.017
interaction	N.S.			N.S.			N.S.			N.S.		
light(poor)	0.934	46.85	**<0.0001**	0.000526	0.0136	0.99	1.31	96.78	**<0.0001**	0.265	39.42	**<0.0001**
total mass	0.0169	1.697	0.20	0.529	0.5295	0.47	0.165	24.43	<0.0001	0.0144	4.304	0.042
interaction	0.116	5.804	**0.0040**	0.311	0.3110	0.74	N.S.			N.S.		
light(intermediate)	0.858	50.61	**<0.0001**	0.106	13.49	**<0.0001**	1.31	112.0	**<0.0001**	0.179	33.77	**<0.0001**
total mass	0.00339	0.3994	0.53	0.0712	18.07	<0.0001	0.183	31.30	<0.0001	0.00853	3.226	<0.077
interaction	0.218	12.83	**<0.0001**	N.S.			N.S.			N.S.		
light(high)	1.70	97.48	**<0.0001**	0.127	3.548	**0.034**	1.10	74.46	**<0.0001**	0.181	32.92	**<0.0001**
total mass	0.106	12.16	0.0007	0.103	5.788	0.019	0.137	18.66	<0.0001	0.00134	0.490	0.49
interaction	N.S.			N.S.			N.S.			N.S.		

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Table 4. ANCOVAS of effect of soil and light on whole-plant allocation Significant main effect and interaction p values in **bold**. NS = not significant.

| | Lateral root mass X Leaf mass | | | | | | Lateral root surface area X Leaf surface area | | | | | |
| | *G. sepium* | | | *H. coubaril* | | | *G. sepium* | | | *H. coubaril* | | |
	SS	F	p	SS	F	p	SS	F	p	SS	F	p
soil(shade)	0.075	0.1703	0.84	0.0575	0.1980	0.82	0.0877	0.1816	0.83	0.0399	0.579	0.56
covariate	5.32	24.12	<0.0001	4.90	33.68	<0.0001	5.68	23.50	<0.0001	0.886	25.71	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		
soil(gap)	0.0762	1.067	0.35	0.0621	0.8137	0.45	0.0275	0.4002	0.67	0.0570	1.439	0.24
covariate	8.481	237.3	<0.0001	6.22	163.1	<0.0001	6.33	184.1	<0.0001	2.46	124.1	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		
soil(open)	1.53	36.51	**<0.0001**	0.589	2.685	0.075	1.24	24.19	**<0.0001**	0.102	1.173	0.3153
covariate	2.79	133.5	<0.0001	9.63	87.80	<0.0001	2.32	90.54	<0.0001	3.27	74.94	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		
light(poor)	8.93	71.57	**<0.0001**	1.35	9.185	**0.0003**	18.5	157.5	**<0.0001**	2.75	42.56	**<0.0001**
covariate	3.20	51.26	<0.0001	7.56	102.9	<0.0001	2.87	48.75	<0.0001	1.93	59.93	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		
light(intermediate)	9.02	38.75	**<0.0001**	3.39	18.65	**<0.0001**	20.5	74.48	**<0.0001**	2.58	42.27	**<0.0001**
covariate	5.94	51.04	<0.0001	3.96	43.51	<0.0001	4.84	35.17	<0.0001	1.55	50.82	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		
light(high)	5.28	29.85	**<0.0001**	0.742	3.086	**0.052**	12.3	64.99	**<0.0001**	1.75	25.0	**<0.0001**
covariate	7.01	79.29	<0.0001	9.86	82.08	<0.0001	6.02	63.48	<0.0001	3.14	90.1	<0.0001
interaction	N.S.			N.S.			N.S.			N.S.		

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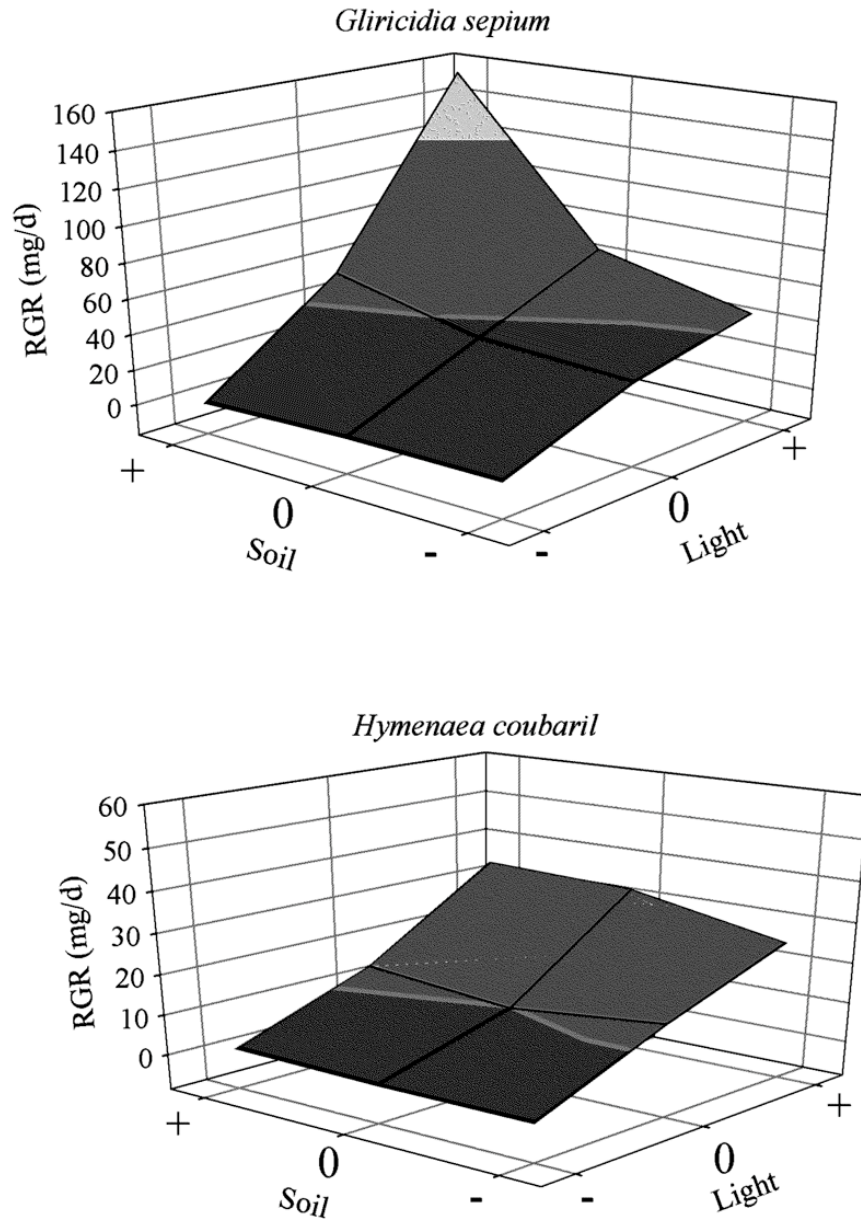
Figures

Figure 1. Response surfaces showing relative growth rates obtained by *G. sepium* and *H. coubaril* across three light and three soil treatments. Symbols -, 0, and + represent shade, gap, and open light treatments and poor, intermediate, and high quality soil respectively.
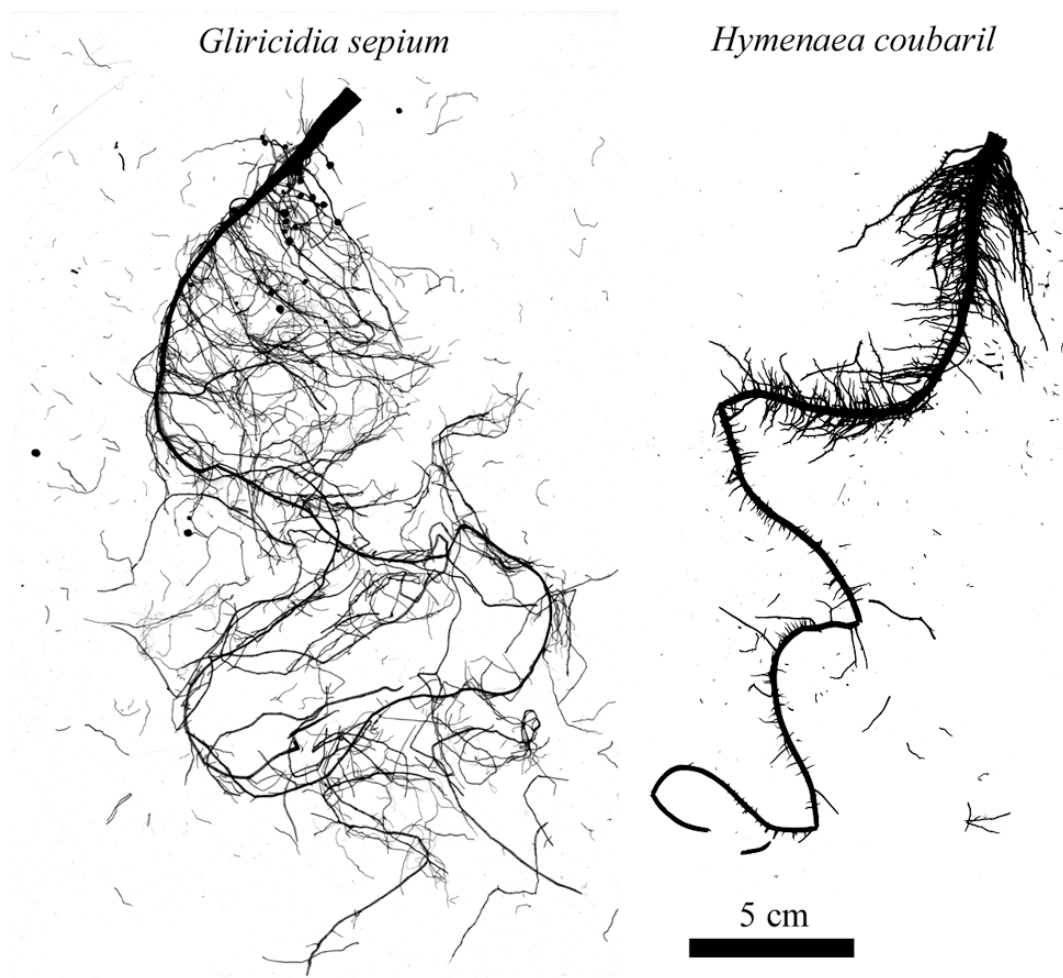
Figure 2. Scans of the root system *G. sepium* and *H. coubaril* to illustrate general differences in morphology. These were not scans from which morphological measurements of the root system were made. Dark circles on the root system of *G. sepium* are nodules.
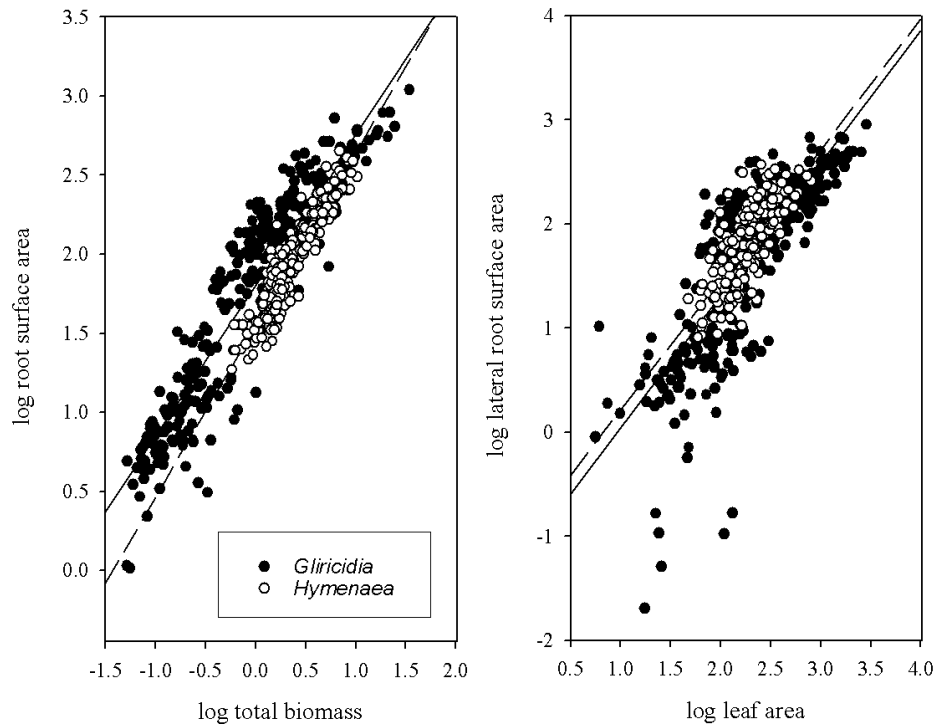
Figure 3. Log-log plots of root system surface area (cm^2) on total plant mass (g) and lateral root surface area (cm^2) on leaf surface area (cm^2), respectively, for *G. sepium* and *H. coubaril*.
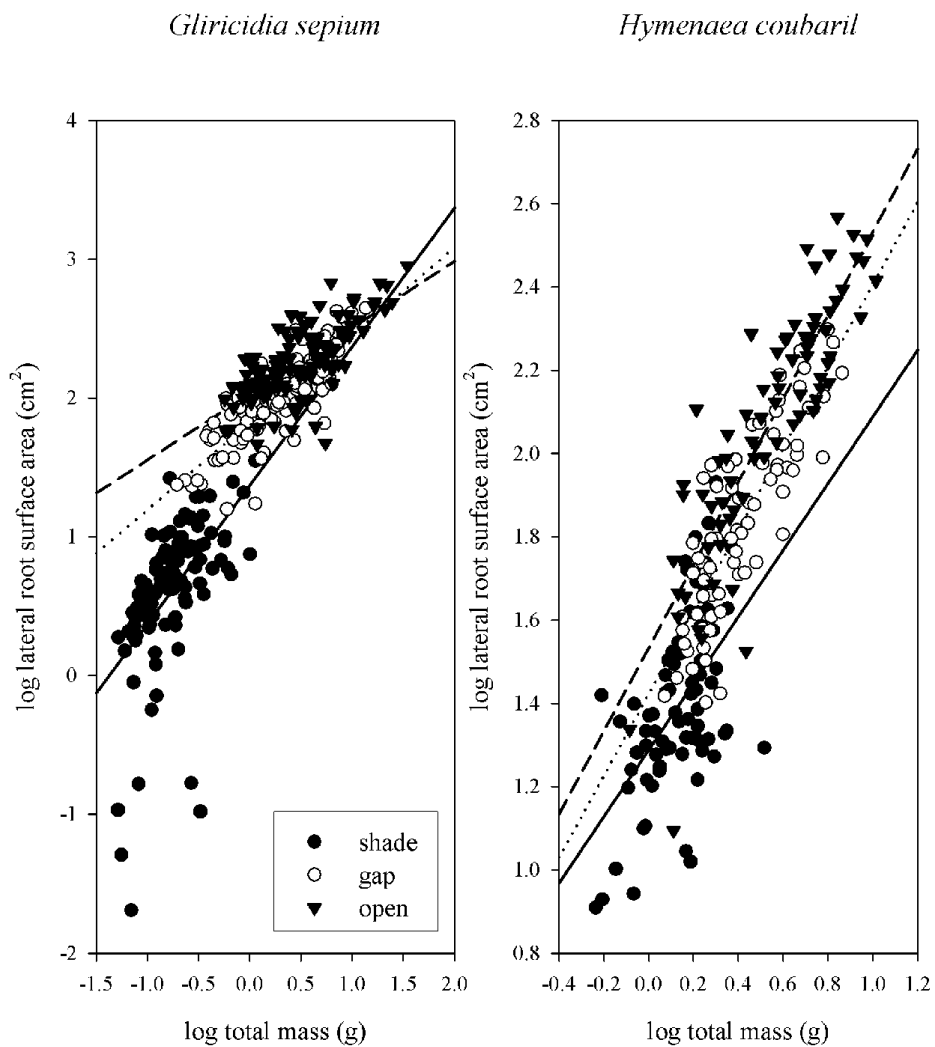
Figure 4. Log-log plot of total lateral root surface area (cm^2) on plant mass (g) in three light environments for *G. sepium* and *H. coubaril*. Lines are least squares regressions calculated individually for each light environment (solid line = shade, dotted line = gap, and dashed line = open light). Comparison is in intermediate soil. Main effects and interactions are significant in both cases.
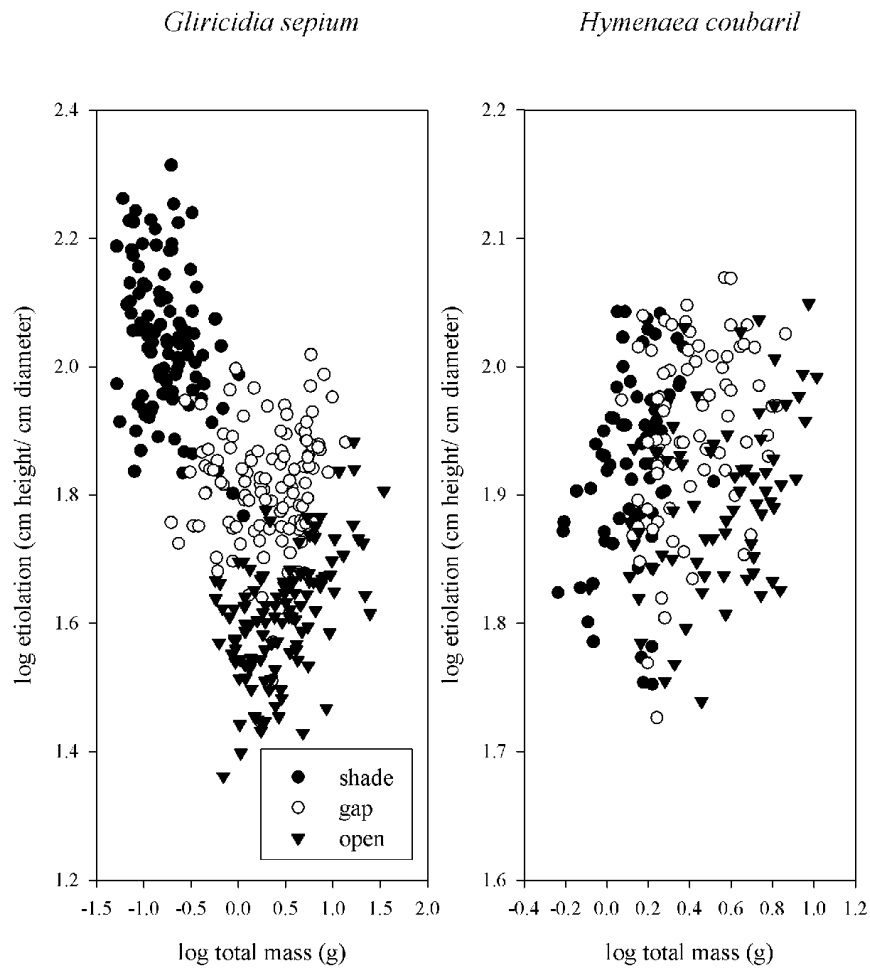
Figure 5. Log-log plot of the etiolation response (plant height/ plant diameter at 5 cm) on total plant mass in three light environments for *G. sepium* and *H. coubaril*. Regression lines are omitted in this case as the covariate explained a small proportion of the variation in the etiolation response. Comparison is in intermediate soil. For *G. sepium* the main effect and interaction are highly significant but the covariate is not. For *H. coubaril* the main effect and covariate are highly significant.

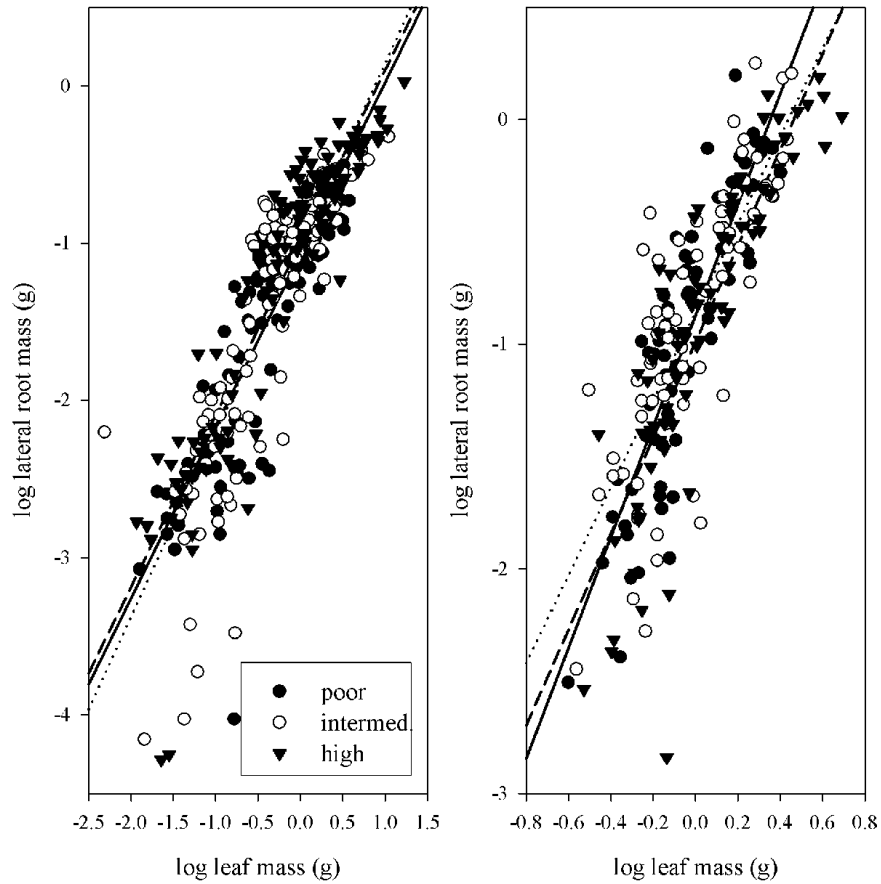*Gliricidia sepium*   *Hymenaea coubaril*

Figure 6. Log-log plot of total lateral root mass (g) on total leaf mass (g) in three soil environments for *G. sepium* and *H. coubaril*. Lines are least squares regressions calculated individually for each light environment (solid line = poor quality, dotted line = intermediate quality, dashed line = high quality soil). Comparison in open light. Main effect is highly significant only for *G. sepium*, with high quality soil having the largest intercept ratio, contrary to the predictions of balanced growth.
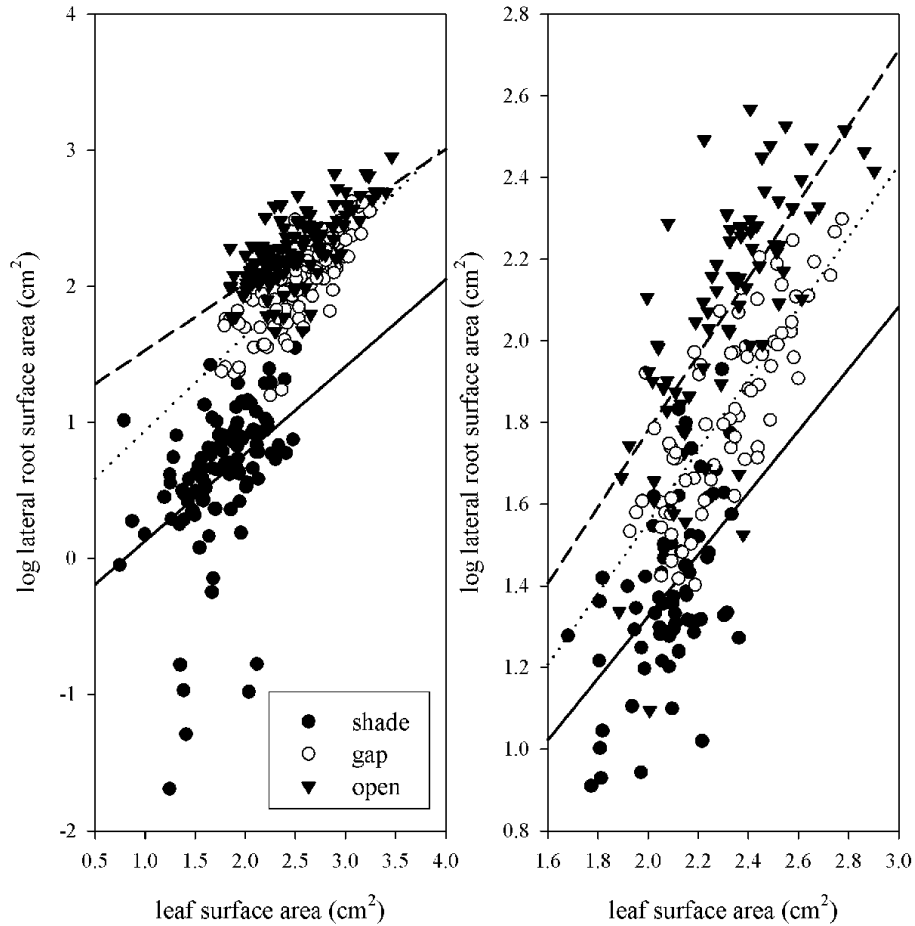
99

Figure 7. Log-log plot of total lateral root surface area (cm²) on total leaf surface area (cm²) in three light environments for G. sepium and H. coubaril. Lines are least squares regressions calculated individually for each light environment (solid line = shade, dotted line = gap, and dashed line = open light). Comparison in intermediate soil. Both the main effect and the covariate are highly significant for both species.