

PLANT BIOMASS ALLOCATION:
UNDERSTANDING THE VARIABILITY WITHIN SIZE CONSTRAINTS

by

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TABLE OF CONTENTS

LIST OF FIGURES.....	9
LIST OF TABLES.....	11
ABSTRACT.....	13
INTRODUCTION.....	15
PRESENT STUDY.....	29
REFERENCES.....	38
APPENDIX A: ORGAN PARTITIONING AND DISTRIBUTION ACROSS THE	
SEED PLANTS: ASSESSING THE RELATIVE IMPORTANCE OF PHYLOGENY	
AND FUNCTION.....	41
Abstract.....	42
Introduction.....	43
Methods.....	47
Statistical analyses.....	49
Results.....	55
Discussion.....	59
Acknowledgements.....	67
Literature cited.....	68
Figure legends.....	74
Tables.....	76
Figures.....	77

TABLE OF CONTENTS – *Continued*

APPENDIX B: CONSISTENCY BETWEEN AN ALLOMETRIC APPROACH AND OPTIMAL PARTITIONING THEORY IN GLOBAL PATTERNS OF PLANT BIOMASS ALLOCATION.....	87
Summary.....	88
Introduction.....	90
Methods.....	94
Results.....	98
Discussion.....	102
Acknowledgements.....	107
Tables.....	108
References.....	110
APPENDIX C: ASSESSING THE RELATIVE EFFECTS OF PHYLOGENY, ENVIRONMENT AND GROWTH FORM ON PLANT BIOMASS ALLOCATION AND LEAF PHENOLOGY.....	117
Summary.....	118
Introduction.....	119
Materials and Methods.....	122
Results.....	126
Discussion.....	127
Acknowledgements.....	131
References.....	132

TABLE OF CONTENTS – *Continued*

Tables.....	138
Figures.....	140
APPENDIX D: TESTING ALLOMETRIC AND OPTIMAL PARTITIONING	
MODELS IN PATTERNS OF BIOMASS ALLOCATION: THE ROLE OF NUTRIENT	
LEVEL AND SPECIES-SPECIFIC DIFFERENCES.....	
Abstract.....	143
Introduction.....	145
Methods.....	150
Results.....	153
Discussion.....	155
Acknowledgements.....	161
References.....	162
Figures.....	167
Tables.....	170

LIST OF FIGURES

APPENDIX A: ORGAN PARTITIONING AND DISTRIBUTION ACROSS THE
SEED PLANTS: ASSESSING THE RELATIVE IMPORTANCE OF PHYLOGENY
AND FUNCTION

- FIGURE 1, Biomass allometric relationships between leaf, stem and root mass for conifers, monocots and dicots from a global compilation of seed plants.77
- FIGURE 2, Allometric relationship between stem mass and total mass minus stem mass viewed across two different size ranges.78
- FIGURE 3, Phylogenetic supertree of seed plant species generated using phylocom.80
- FIGURE 4, The amount of variation in distribution and partitioning explained by each taxonomic level.....81
- FIGURE 5, Relationships between the mean leaf mass residuals and leaf traits for seed plant species.83

APPENDIX B: CONSISTENCY BETWEEN AN ALLOMETRIC APPROACH AND
OPTIMAL PARTITIONING THEORY IN GLOBAL PATTERNS OF PLANT
BIOMASS ALLOCATION

- FIGURE 1, PC2 and PC3 plotted in bivariate space for *Cryptomeria japonica*.....98
- FIGURE 2, The relationships for *Cryptomeria japonica* PC2 and PC3 with environmental variables.....100
- FIGURE 3, PC2 and PC3 plotted in bivariate space for all seed plants101

LIST OF FIGURES – *Continued*

APPENDIX C: ASSESSING THE RELATIVE EFFECTS OF PHYLOGENY,
 ENVIRONMENT AND GROWTH FORM ON PLANT BIOMASS ALLOCATION
 AND LEAF PHENOLOGY

FIGURE 1, Biomass allometric relationships.140

FIGURE 2, The relationship between the age of a node and the amount of variation
 explained by that bifurcation141

APPENDIX D: TESTING ALLOMETRIC AND OPTIMAL PARTITIONING
 MODELS IN PATTERNS OF BIOMASS ALLOCATION: THE ROLE OF NUTRIENT
 LEVEL AND SPECIES-SPECIFIC DIFFERENCES

FIGURE 1, Allometric scaling relationships between the log of above ground
 biomass to the log of below ground biomass for 20 species of Eudicots grown in
 two different nutrient levels.....167

FIGURE 2, Interspecific allometric scaling relationship between the log of above
 ground biomass to the log of below ground biomass for 20 species of Eudicots
 grown in two different nutrient levels.....169

LIST OF TABLES

APPENDIX A: ORGAN PARTITIONING AND DISTRIBUTION ACROSS THE SEED PLANTS: ASSESSING THE RELATIVE IMPORTANCE OF PHYLOGENY AND FUNCTION

TABLE 1, Variation for Stem Mass, Leaf Mass and Leaf Annual Production due to Growth Form, Phylogeny and Phylogenetically Structured Growth Form.....	76
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APPENDIX B: CONSISTENCY BETWEEN AN ALLOMETRIC APPROACH AND OPTIMAL PARTITIONING THEORY IN GLOBAL PATTERNS OF PLANT BIOMASS ALLOCATION

TABLE 1, PC2 Eigenvector loadings for leaf, stem and root mass from principal component analyses for <i>Cryptomeria japonica</i> and all seed plants....	108
TABLE 2, The predicted direction and results of correlations between PC 2 and 3 for <i>Cryptomeria japonica</i> with the log of precipitation, relative humidity and sunlight and decreased water stress. ...	109

APPENDIX C: ASSESSING THE RELATIVE EFFECTS OF PHYLOGENY, ENVIRONMENT AND GROWTH FORM ON PLANT BIOMASS ALLOCATION AND LEAF PHENOLOGY

TABLE 1, Eight genera of Eudicots each containing two species of annual, perennial and woody perennial growth forms....	138
TABLE 2, Amount of variation in leaf mass, stem mass, root mass, specific leaf area, photosynthetic potential and LAR explained by growth form, nutrient level	

LIST OF TABLES – *Continued*

and phylogeny.....	139
APPENDIX D: TESTING ALLOMETRIC AND OPTIMAL PARTITIONING MODELS IN PATTERNS OF BIOMASS ALLOCATION: THE ROLE OF NUTRIENT LEVEL AND SPECIES-SPECIFIC DIFFERENCES	
TABLE I, Species from each Genus Separated into Annual, Perennial and Woody Perennial Growth Forms....	170
TABLE II, Percent Variation in Biomass Allocation explained by Increased Nutrient Level.....	171
TABLE III, Percent of Variation in Total Size and Leaf Traits explained by Nutrient Level... ..	173
TABLE IV, Percent of Species that Demonstrated the Predicted Response for each Trait... ..	174

ABSTRACT

The majority of studies on plant biomass partitioning have focused on the effects of environment. Optimal Partitioning Theory (OPT) suggests that plants should allocate biomass to the organ that acquires the most limiting resource. Though, it has recently been disputed as to how much of this variation is due to variation in size and not environment. Additionally, while a few studies have examined differences between growth forms, the effects of evolutionary history have been largely ignored. Leaf morphology and physiology may also contribute to patterns of biomass partitioning.

The role of plant size has been shown to be considerable to plant biomass allocation. Allometric biomass Partitioning Theory (APT) has recently been proposed to predict how plants should partition metabolic production based on the constraints of body size. Here, I assess the relative contribution of environment, growth form, leaf traits and phylogeny on variation in biomass allocation, after accounting for changes in size, using both an empirical and experimental approach. I use a global dataset of seed plants in addition to growing plants with differing evolutionary histories and growth forms hydroponically in two nutrient levels to examine patterns of organ partitioning while accounting for allometrically driven biomass allocation. Both the empirical and experimental interspecific analyses indicate that phylogeny accounts for the majority of the variation in biomass partitioning. Leaf biomass partitioning is partially related to growth form, however this appears to be due to differences in leaf morphology and physiology. While a strong phylogenetic signal exists, about half of the variation was not explained by any of the factors interspecifically, suggesting room for plasticity in

partitioning. Intraspecifically, biomass allocation and partitioning was related to environmental factors in the directions predicted by OPT. However, the species-specific allocation response to environmental differences was not uniform, therefore obscuring interspecific patterns. These results have important implications for ecological studies; such that partitioning studies must first assess the role of plant size *and* evolutionary history in order to fully understand variability in biomass partitioning. Additionally, differences from environment can be incorporated with allometric changes to help understand how plants should allocate biomass.

INTRODUCTION

Plants have a limited amount of energy to divide between the multiple functions an individual must perform. Plants gain energy through photosynthesis, which they can put into root, stem or leaf mass, maintenance, storage, defense or reproduction. The diversity of plant growth forms suggests there are many ways that plants can partition the energy that is put into biomass. The question of how plants partition biomass has been examined for almost a century, with the majority of theories and approaches to this research being around almost as long. The major areas of study for biomass partitioning have been how the environment, allometry, and species differences influence the amount of biomass that is allocated to roots relative to shoots, although seldom have these ideas been used together. Roots and shoots have been the focus of biomass partitioning studies because of the two main functions a plant must perform to survive and grow; plants must be able to absorb nutrients and water from the soil, which occurs in the roots, and they must be able to harvest carbon dioxide to make sugars, which occurs in the shoots.

Researchers have been interested in how plants partition biomass for multiple reasons. Some of the early studies were interested in how different conditions affected biomass partitioning for the purpose of manipulating agricultural crops (Crist and Stout 1929, Luckwill 1960). Most studies are interested in how plants partition biomass because form is related to function, so biomass partitioning should be related to plant fitness. Many experimental studies use relative growth rate to determine how plants should partition biomass (Hunt and Lloyd 1987, Davidson 1969) while some theoretical studies assume plants partition biomass in a way that maximizes their lifetime

reproductive output, which can be a difficult criteria to use in an experimental setup. Partitioning of biomass within an individual plant can have implications beyond plant fitness. Increases in the ratio of root to shoot biomass have been found for plants during subsequent successional stages, suggesting that biomass partitioning may be important for ecological dynamics (Monk 1966). Relative allocation to shoot versus root will also affect total above ground standing crop, which affects ecosystem processes. Each habitat may favor a different allocation pattern due the level of soil resource and light level and the loss rate from herbivory and disturbance, which all select for a particular plant form (Tilman 1988). Therefore, as humans change the environment plants are exposed to, it is important to understand how these changes will affect how much biomass plants put into carbon sequestering relative to absorption.

A large amount of the research on biomass partitioning has looked at environmental influences. Crist and Stout (1929) increased interest in this area with their studies on crop plants, where they demonstrated that plants can alter their biomass partitioning dramatically in response to environmental variation. When lettuce, tomato and radish plants were placed under different soil, nutrient, light, container size, pruning, and reproductive removal treatments, shoot to root ratios varied from 0.79 to 12.96. Increased nutrients, shortened daylength and larger pot size all caused an increase in the shoot to root ratio. Pruning plants and not removing flowers also produced an increasing trend in shoot to root ratio, but the results were small and insignificant. This study and many studies after it show a consistent trend, which became known as optimal partitioning theory, of plants allocating more biomass towards the organ that gathers the

most limiting resource (Crist and Stout 1929). For instance, if nutrients or water are limiting, plants allocate more biomass to roots and if light or CO₂ is limiting, plants should allocate more to shoots.

Subsequent studies revealed that changes in biomass partitioning due to environmental effects may be related to changes in the photosynthetic and absorption rates of the shoots and roots. Because absorption and photosynthesis are thermodynamic processes, they should be affected by light, temperature, and resource supply (Davidson 1969). Luckwill (1960) proposed that photosynthate was partitioned inversely proportional to root absorption and shoot photosynthesis. Davidson (1969) tested whether root mass * root absorption is proportional to leaf mass * leaf photosynthetic rate by placing 12 pasture species under different soil temperatures and comparing their growth rate to their root to shoot ratio. The optimal soil temperature was determined by the maximum foliage yield, which is assumed to be related to optimal root activity, and soil temperature was compared to the root to shoot ratio. The root to shoot ratio was lowest at the temperature optimum, the temperature at which roots should have maximum absorption, and increased both above and below the temperature optimum, indicating that root partitioning is lowest at the highest root activity and lowers with increasingly suboptimal absorption rates (Davidson 1969). This result also indicates that biomass partitioning should vary seasonally as soil temperature changes, indicating that it may be important to consider seasonality in biomass partitioning studies that compare plants in different environments. Furthermore, leaf morphology, which is related to photosynthetic rates (Reich et al. 1997), may also be important in allocation patterns.

The previous studies show intriguing results regarding how dramatically the environment can change plant biomass partitioning, however they fail to take size differences into account. It has been recognized for over a century that roots and shoots are mutually dependent on each other (Kny 1894). Kny (1894) examined how trimming shoots of *Zea mays* seedlings would affect root growth and vice versa. The results showed that when shoots were removed, root mass was greatly reduced and when roots were removed shoot mass was reduced.

This is expected because shoots depend on the roots to absorb nutrients and water and the roots need organic compounds that the shoots supply. Therefore an increase in the size of one organ allows an increase in size in the other. However, if these organs are increasing at different rates, a divergence in size will occur over time, therefore changing the root to shoot ratio. Brenchley (1920) found that for peas grown in a water culture, shoot mass was greater than root mass during all stages of growth. The difference in size increased during development because shoots grew more rapidly than roots, regardless of nutrient supply, causing the shoot to root ratio to increase. The divergence in size was reportedly due to roots needing only fine root hairs to increase absorption, while shoots need to increase a greater bulk of tissue to produce a larger surface area.

Allometry examines how plant form changes with size, and can be used to see how root mass changes in relation to shoot mass. Root mass can be plotted against shoot mass in log-log space such that $\log Y = \log c + k \log X$ where Y and X are root and shoot mass respectively, c is the y-intercept and k is the slope. If k is equal to one, for every increase in shoot mass, root mass increases proportionally. If k is greater than or less

than one, however, root mass increases faster or slower than shoot mass. If root mass and shoot mass are not changing proportionally, an increase in mass could cause a higher or lower root to shoot ratio, which has nothing to do with environmental or species-specific differences.

Pearsall (1927) suggested that if the root and shoot of a plant are both growing, the log of the shoot mass should be proportional to the log of root mass during any particular point of plant development. This should occur because the shoot and root weight (S_t and R_t respectively) are a function of their initial weights (S_o and R_o respectively), growth rates (x and y respectively) and time (t), such that

$$S_t = S_o e^{xt} \text{ and } R_t = R_o e^{yt} \text{ and } S_o = cR_o.$$

Therefore S_t , R_t , S_o , and R_o are all related and the relationship between root and shoot becomes $S_t = cR_t^{x/y}$ or if you take the log of both sides $\log S = \log c + k \log R$ where $\log c$ is the intercept and $k = x/y$. The relationship between root and shoot mass for cotton, etiolated pea, normal pea, carrot and turnip plants, under various environmental conditions, produced a linear relationship in log-log space with slopes (k) ranging between 0.55 to 2.65 for 16 relationships. When environmentally manipulated plants were removed the slopes ranged between 0.55 to 1.3 where “the values of k clearly range round unity” (Pearsall 1927). Several species had very similar slopes but varied in their y-intercepts, however fleshy root plants, such as carrots, had lower slopes. Pearsall (1927) proposed that $k=1$ suggested similar growth efficiencies for the root and shoot, which may be due to the stem and root meristem being derived from the same protoplasm and the dividing zone being a volume for both the root and shoot. If the shoot

was growing as an area, but the root as a volume, a slope value of 0.66 would be expected.

Niklas and Enquist (2002) suggested a different reason that the slope from the allometric relationship of log root mass on log shoot mass should have a slope of one. Based on empirical observations and theory, a series of scaling relationships were derived to explain how plants should partition biomass. Leaf mass is predicted to scale isometrically to metabolic activity because leaves are the main photosynthetic organ. Metabolic activity has been shown both theoretically and empirically to scale to the $3/4^{\text{th}}$ power of body size, therefore, leaf mass should also scale to the $3/4^{\text{th}}$ power of body size. Then, to conserve the flow of water through the plant, the length, diameter and density of the stem and root should be proportional. So under these assumptions leaf mass should be proportional to the $3/4^{\text{th}}$ power of stem and root mass. This prediction was tested using a world-wide forestry database that contained arboreal dictots, monocots and conifers, in addition to biomass data for herbaceous species and woody seedlings gathered from the primary literature. The data fit the prediction remarkably well with the exception that root mass tended to scale slightly lower than predicted and shoot mass scaled slightly higher, which may be due to an underestimation of fine root mass for larger individuals. Within the constraint of body size, there was one order of magnitude variation in root mass for a given shoot mass and one to two orders of magnitude variation of leaf mass, with gymnosperms tending to have more leaf mass than angiosperms for a given size. The scatter within these relationships may account for species-specific differences and habitat. The difference in leaf mass between

gymnosperms and angiosperms reflects the differences in leaf phenology between these two clades. Gymnosperm leaves have lower photosynthetic rates, due to lower specific leaf area (Ackerly and Reich 1999), but have longer life spans. This allows gymnosperms to keep leaf cohorts longer and increases the amount of leaf mass (Niklas and Enquist 2002). This trend also supports Davidson's (1969) hypothesis that plants may compensate in decreased activity of an organ by increasing the mass. Other differences between species in organ traits may account for much of the variation in the y-intercept.

Allometry may explain much of the variation found in biomass partitioning that would be explained only by environmental or species effects if size was not considered. Coleman and McConnaughay (1995) reexamined data from an earlier study by Mooney et al. (1988) that suggested SO_2 decreased photosynthetic activity in radish shoots, but increased partitioning to leaf material to compensate. But when Coleman and McConnaughay (1995) compared the root to shoot ratio and the natural log of root mass to the natural log of total biomass, the differences between plants not exposed and exposed to SO_2 disappeared, indicating the differences in relative allocation were due to differences in growth rate, which created differences in size (Coleman and McConnaughay 1995).

McConnaughay and Coleman (1999) looked further into how much optimal partitioning due to environmental variation affected biomass partitioning compared to size differences by varying light, water and nutrients for three annual species. When size differences were not taken into account, i.e. root to shoot ratios were compared to time,

biomass partitioning was affected for at least two of the species with all three environmental variables. When root to shoot ratio was compared to the log of body size, partitioning differences due to light and nutrient levels were still present, although slightly smaller, however water level variation did not affect root relative to shoot allocation. When four fast growing annual species were grown in different carbon dioxide levels (Bernacchi et al 2000), ambient (350 ppm) and future predicted (700 ppm), some species had lower root to shoot ratios in elevated CO₂; this is the opposite of what would be expected under optimal partitioning theory, which would predict more mass would be allocated to the roots when shoots had a high CO₂ supply. But when root to shoot ratio was compared to the log of body size, these differences disappeared (Bernacchi et al 2000). These studies indicate that plants do not always change their partitioning strategies in response to differing environmental factors.

Other studies indicate that differences in biomass partitioning due to environment can still be quite large after size is considered. Log root mass was compared to log shoot mass for *Lolium perenne* placed under three light and two potassium levels (Hunt and Burnett 1973). Potassium levels did not affect biomass partitioning, however slope and y-intercept both increased with each increase in light level, supporting optimal partitioning theory which would predict an increase in root mass partitioning with increasing light. This study also provided support for Davidson's (1969) theory that root mass * root absorption is proportional to leaf mass * leaf photosynthetic rate. Rearranging this equation illustrates that root mass/leaf mass should be proportional to leaf photosynthetic rate/root absorption rate. In Hunt and Burnett's (1973) study, root to

shoot ratio was positively related to the reciprocal of root and shoot activity ratio (shoot activity/ root activity) with a correlation coefficient of 0.947, supporting the theory that plants adjust biomass allocation to compensate for lowered activity rates.

Species-specific differences in growth form also have an effect on how much biomass is allocated to roots relative to shoots. In a comparison of the log of shoot mass to the log of root mass for nine old-field herbaceous species and six woody seedlings, spanning four orders of magnitude in shoot size, species with different growth forms had similar slopes but varied in their y-intercept (Monk 1966). Woody perennials had the highest y-intercept, and therefore the highest root to shoot ratio for a given size, followed by herbaceous perennials, and subsequently annuals, with woody perennials and annuals differing by an order of magnitude. This variation is close to the variation found by Niklas and Enquist (2002), which spanned ten orders of magnitude and contained several hundred species, suggesting that much of the variation found in biomass allocation may be due to species-specific differences.

Tilman (1988) proposed that species-specific differences in root versus shoot allocation have consequences on their growth rate. According to Tilman's model, species with higher root to shoot ratios will have lower growth rates because both photosynthetic and non-photosynthetic material respire (model assumes equal respiration rates), but non-photosynthetic material does not fix carbon so more biomass going towards roots would cause a reduction in growth rate. This hypothesis was tested by comparing the relative growth rate to the root to shoot ratio or the allometric coefficient for log root mass compared to log shoot mass for 68 species of herbaceous

angiosperms (Shipley and Peters 1990). Relative growth was positively correlated to both the root to shoot ratio, at 30 days, and the allometric coefficient, which is the opposite of what Tilman's model would predict. Hunt and Lloyd (1987) also found that species-specific differences in allometric coefficient were positively related to the mean relative growth rate, although the relationship was not extremely tight. Faster growing species allocated more to root mass, but slow growing species could allocate more mass to either roots or shoots. This trend is the opposite of Monk's (1966) findings, which demonstrated that annuals, which are fast growing, had the highest root to shoot ratio for a given body size. Further investigation may be necessary to determine how biomass allocation is related to species functional traits such as growth rates.

It appears that size, life history differences and environmental changes can all affect how plants partition biomass. Given all of these effects on biomass partitioning, it would be useful to know how these effects interact. Do species-specific differences wash out when environmental change is considered? Hunt and Nichols (1986) addressed this issue by examining how three different nutrient and light levels affected the allometric coefficient for three grasses with different growth rates. The slow growing species was representative of a stress tolerator, the species with medium growth corresponded to a species that tolerated low stress and moderate disturbance, and the fast growing species represented a species that tolerated high disturbance and low stress. For all three species, the allometric coefficient increased with increasing nutrient stress and decreased with increasing light stress, however nutrient stress had a greater effect on the allometric coefficient than light stress so that nutrient plus light stress resulted in a higher allometric

coefficient than for no stress. Although the allometric coefficients for all of the species changed in the same direction for a given stress, the magnitude of the change varied between the species. Slower growing species had greater changes in their allometric coefficients in addition to having higher growth rates relative to their maximum growth rate, than faster growing species, indicating that species with differing life history traits can vary in their biomass partitioning behavior.

Most of the studies of biomass partitioning have concentrated on root and shoot mass due to the two main functions a plant performs: photosynthesis and absorption. Yet not all root mass and shoot mass are equivalent in their abilities to perform these two functions and there are many functions a plant must perform. Because the primary goal with biomass partitioning studies is to address how plant form affects plant function, should we be dividing plant biomass based on the function of the various components instead of the morphology? Korner (1994) suggests plant biomass should be separated by function such that there is a photosynthesis function which contains leaves, an absorption function which contains fine roots, and another category that includes a support and transport function containing stems, leaf petioles and coarse roots, a reproductive function containing fruits and associated structures, a storage function, and an export function. One shortcoming of this approach is that organs can perform multiple functions. Fruits and stems can photosynthesize in addition to their main functions. In addition, below and above ground stem assists the plant in different ways; the above ground stem allows a plant to get better access to light, and therefore aids in photosynthesis, while below ground stem/ coarse roots allow the fine roots to get deeper

can get further away, which aids the plant in getting water and nutrients. Korner (1994) implemented this approach by examining leaf mass to fine root mass ratio for plants from several life forms ranging from floating water plants to conifers and deciduous trees. All varied in the percent of leaf mass the individuals contained; floating water plants had the highest percent leaf mass followed by annuals, biennials, perennials, shrubs, and trees had the lowest percent leaf mass. However, these groups also differed markedly in size. Because leaf mass appears to scale to the $3/4^{\text{th}}$ power of body size (Niklas and Enquist 2002), larger individuals, such as trees, would be expected to have a higher percent leaf mass than smaller individuals, such as herbaceous perennials and annuals. Korner (1994) also found that conifers had around three times more leaf mass than deciduous trees, but this was also found by Niklas and Enquist (2002). Although Korner's biomass division based on function appears to be a more descriptive way of separating biomass than the simple root versus shoot division, it may not be any more informative than dividing biomass into root, leaf and stem division. Additionally, since functional attributes of organs vary depending on the species and fine roots are extremely difficult to measure accurately, the root stem and leaf division may be the simplest method while still providing meaningful information. A root, leaf and stem division addresses the fact that stems do not perform the same function as leaves, while maintaining a system that is usable across a large diversity of species.

Species differences, nutrient availability, water availability, light intensity and seasonality have all been shown to alter biomass partitioning (Hunt and Burnett 1973, Hunt and Nichols 1986, Davidson 1969, Monk 1966, Iwasa 2000). After body size is

accounted for, there are one to two orders of magnitude variation in biomass partitioning left to be explained by these variables (Niklas and Enquist 2002). Both species-specific differences and environmental variation have been shown to affect biomass partitioning, however it is not clear how these variables may fit into the variation left from large scale allometric comparisons. Differences between species in life history characteristics appear to change the y-intercept (Monk 1966), yet in other studies, differences between species in biomass allocation affect the allometric coefficient (Hunt and Lloyd 1987, Shipley and Peters 1990). Environmental variation also seems to have an effect on the allometric coefficient instead of causing differences in the y-intercept (Pearsall 1927, Hunt and Nichols 1986, Hunt and Lloyd 1987). However, if this is the case, it suggests that large-scale environmental change may affect the entire allometric relationship demonstrated by Niklas and Enquist (2002), instead of simply the residual variation within the existing allometric relationship for seed plants. This seems unlikely since the plants used to form the relationship (Niklas and Enquist 2002) were from a world-wide forestry database. An alternative reason for the deviations in allometric coefficients in many biomass partitioning studies is a lack of size variation, which would cause extreme sensitivity in the slope. For studies using only a couple of species, it is difficult to obtain enough size variation to get a stable regression. A large enough size variation is needed to form a stable regression, and then variation within that regression can be used to analyze other factors that influence biomass partitioning patterns. The partitioning relationships found by Niklas and Enquist (2002) will provide the opportunity to

determine how the residual variation from the biomass partitioning patterns is affected by environmental conditions and species differences.

This dissertation addresses how environmental variation, growth form, leaf morphology and physiology and evolutionary history affect variation in biomass partitioning after accounting for differences in size. Plants appear to be operating within certain constraints due to size (Niklas and Enquist 2002), but within this constraint, are there consistent trends in how the environment affects biomass partitioning that apply to all or most species? Or do species not fall under a general pattern of biomass partitioning due to differences in growth form, leaf morphology or simply evolutionary history? By using a global dataset of seed plants in addition to an experimental set up of 48 species spanning the eudicots and three growth forms grown hydroponically in two nutrient levels, I address how size, environment, growth form, leaf traits and evolutionary history all contribute to biomass partitioning patterns. Determining how the environment and species differences fit into the constraint of body size may provide valuable predictive power to understand differences in plant biomass partitioning patterns. These patterns will be important to understand in order to obtain a predictive relationship of how global warming, increasing CO₂ and other types of environmental change may affect plant form.

PRESENT STUDY

The methods, results and conclusions of this dissertation are presented in appended manuscript form. The main results and conclusions are summarized below.

ORGAN PARTITIONING AND DISTRIBUTION ACROSS THE SEED PLANTS:

ASSESSING THE RELATIVE IMPORTANCE OF PHYLOGENY AND FUNCTION

(This manuscript, appendix A, has been accepted to *The International Journal of Plant Sciences*, but has not yet been published. The main methods, results and conclusions are below.)

The majority of studies on biomass partitioning are centered around Optimal Partitioning Theory (OPT), which suggests that plants should allocate biomass to the organ that acquires the most limiting resource. However, recent studies demonstrate that the differences previously attributed to environment are largely due to measuring biomass at a common time instead of a common size. Some studies have also examined the effects of differing growth forms (Monk 1966; Tilman 1988), however the effects of evolutionary history have been relatively ignored. This study addresses the relative importance of environment, growth form, leaf morphology and physiology and phylogeny on patterns of biomass allocation after accounting for differences in size.

This study uses a global dataset of arboreal monocots, dicots and conifers (Cannel 1982). Additionally, herbaceous species and seedlings have been added to broaden the size range (Enquist and Niklas 2002). Furthermore, I have added growth form (USDA 2002), taxonomic and phylogenetic information (Stevens 2001 onwards), leaf traits

(Wright et al. 2004) and environmental conditions (New et al. 2002) for each species and location. While plant size explained 97 to 99% of the variation in biomass distribution to leaf, stem, and root mass (Enquist and Niklas 2002), there exists one to two orders of magnitude residual variation around these allometric scaling relationships. I used the residual variation around these scaling relationships, the variation not explained by size, to determine the relative contribution of evolutionary history, growth form, leaf traits and environment to biomass allocation. I further examined whether partitioning patterns are evolutionarily constrained and the specific effect of leaf morphology and physiology.

Evolutionary history was related to over a third of the variation in leaf, stem and root biomass partitioning and distribution. The most notable difference was in leaf mass allocation between Angiosperms and Gymnosperms. Gymnosperms have higher leaf mass for a given body size, which is not due to higher annual leaf mass production. Furthermore, despite the higher leaf mass, Angiosperms have higher annual production, leading to a larger annual production per unit leaf mass in Angiosperms. This divergence is explained by large differences in leaf morphology and physiology between these clades. Gymnosperms, in general, have much lower specific leaf areas (SLA), the amount of leaf area for a given leaf mass, than Angiosperms. This difference in leaf morphology is related to physiological traits (Reich et al. 1997). Plants with higher SLAs also have higher photosynthetic potentials and lower leaf lifespans. These differences lead to plants with low SLAs, such as needle-leaf conifers, incurring greater leaf mass, based on the accumulation of long lived leaves, while having lower photosynthetic rates, leading to lower annual production per unit leaf mass.

These results indicate that evolutionary history is an important component of patterns in biomass partitioning when looking globally across large body size ranges. In addition, morphological and physiology differences in leaf traits between species leads to predictable changes in leaf mass allocation. Thus, by using the allometric models as a baseline to understand residual variation in biomass partitioning, in addition to incorporating differences due to leaf morphology and physiology into the allometric coefficients of the biomass partitioning model, we can obtain a more detailed understanding of processes influencing variation in organ partitioning across the Spermatophytes. Understanding differences in organ partitioning must first assess the role of plant size *and* evolutionary history. Future studies that incorporate phylogeny, size, function, and environmental conditions together will likely provide rich insight into the ecological and evolutionary processes that shape differences in botanical biomass partitioning and allocation.

CONSISTENCY BETWEEN AN ALLOMETRIC APPROACH AND OPTIMAL PARTITIONING THEORY IN GLOBAL PATTERNS OF PLANT BIOMASS ALLOCATION.

(This manuscript, appendix B, has been accepted to *Functional Ecology*, but has not yet been published. The main methods, results and conclusions are below.)

Understanding how plant biomass is distributed between roots, stems and leaves is central to many questions in life history evolution, ecology, and ecosystem studies. Current ecological dogma suggests that patterns of biomass partitioning mainly result

from differences in environmental conditions. These studies are centered about Optimal Partitioning Theory (OPT) which suggests that plants should allocate biomass to the organ that acquires the most limiting resource. However, the generality of OPT has recently been drawn into question. These studies demonstrate that the differences previously attributed to environment are mainly due to divergences in size. Additionally, the importance of evolutionary history in biomass distribution has not been taken into account in the majority of these studies. An alternative approach, Allometric biomass Partitioning Theory (APT), has recently been developed to predict how plants should divide biomass between leaves, stems and roots based on the constraints of body size. This study examines the variation in biomass partitioning for a global dataset of seed plants to determine whether OPT occurs within the constraints due to size.

This study uses a global dataset of arboreal conifers, monocots and dicots to determine whether patterns predicted by OPT fit into large-scale size constraints. Climate data (New et al. 2002) for each species at each location was added to the database. Climatic variables includes monthly measures for precipitation (mm/month), number of wet days (number of days with >0.1 mm/month), mean temperature (degrees Celsius), mean diurnal temperature range (degrees Celsius), relative humidity (percent), sunlight (percent of day length with full sunlight) and ground-frost (number of days with ground frost per month) (New et al. 2002). A Principle Components Analysis was used to determine the predominant tradeoffs in allocational patterns. The first principle component axis has equal loadings for stem, root and leaf mass, essentially showing an increase in size, and was therefore not used in the analyses. However, the second and

third PC axes accounted for natural tradeoffs between these organs, corresponding to tradeoffs in plant function. The second PC axis represents a tradeoff between photosynthetic and non-photosynthetic mass and the third represents a tradeoff between above and below-ground function.

The relationship between environment and biomass partitioning was examined for *Cryptomeria japonica*, the best represented species in the dataset, as well as for all seed plants, using independent contrasts (Webb et al. 2004). *Cryptomeria japonica* showed all of the correlations between the biomass partitioning axes and environmental factors that would be predicted by OPT; leaf mass and above ground biomass increased with increased availability of water at the expense of root mass, while the opposite occurred with increases in light availability. However, interspecifically, only one of these relationships existed and was also greatly reduced.

Although very little support was found for OPT, after size was accounted for, at the interspecific level, intraspecifically patterns predicted by OPT were well supported. These results that variation caused by environmental factors can be integrated with the changes related to size to understand how plants should allocate biomass. However, while optimal partitioning is observed, patterns may vary between species making large-scale interspecific patterns unclear. This may be a result of plants being able to alter several physiological and morphological factors in response to the environment, suggesting the need to include a suite of traits to examine in response to environmental change.

ASSESSING THE RELATIVE EFFECTS OF PHYLOGENY, ENVIRONMENT AND GROWTH FORM ON PLANT BIOMASS ALLOCATION AND LEAF PHENOLOGY

(This manuscript, appendix C, has been submitted to *New Phytologist*, and is currently in review. The main methods, results and conclusions are below.)

Understanding how plant biomass is distributed between roots, stems and leaves is central to many questions in life history evolution, ecology, and ecosystem studies. While most previous and current studies have mainly considered the effect of environmental variability, there are several other factors that may contribute to patterns of biomass partitioning. Differences in plants size account for much of the variation in biomass partitioning patterns (Enquist and Niklas 2002). Additionally, growth form has been shown to alter biomass allocation (Monk 1966; Tilman 1988), and evolutionary history is important to several areas of plant form and function (Antunez et al. 2001; Brouat et al. 1998), though it has not been examined extensively for biomass allocation (Osone and Tatenno 1995). This study determines the relative contribution of growth form, phylogeny and nutrient differences to patterns of biomass partitioning.

The relative contribution of growth form, evolutionary history and nutrient level was examined for 48 species of eudicots, consisting of three different growth forms, grown hydroponically in two nutrient levels. The program Phylocom (Webb et al. 2004) was used to determine the evolutionary distance between each of the taxa examined. An extra sum-of-squares F test was used to determined how much phylogeny, growth form and nutrient level contributed to the variation not explained by size interspecifically.

Phylogeny was related to about half of the variation in leaf, stem and root biomass partitioning, however growth form was not significantly related to variation in any of the organs. Nutrient level did not explain any of the variation for stem mass, but explained a small amount of variability for leaf and root mass interspecifically. The strong phylogenetic signal was not due to trait conservatism, but rather appeared to be the result of recent divergences for stem and root mass.

The importance of body size to plant form and function has become increasingly apparent (Niklas 1994; Enquist and Niklas 2002; Niklas and Enquist 2002). Despite the emphasis on environmental effects (Davidson 1969; Hunt and Burnett 1973; Hunt and Nichols 1986), these results demonstrate a strong phylogenetic signal in biomass partitioning. These results suggest that a large-scale understanding of plant biomass allocation across diverse groups must consider the effects of both phylogeny and plant size.

TESTING ALLOMETRIC AND OPTIMAL PARTITIONING MODELS IN PATTERNS OF BIOMASS ALLOCATION: THE ROLE OF NUTRIENT LEVEL AND SPECIES-SPECIFIC DIFFERENCES.

(This manuscript, appendix D, has been submitted to *Oecologia*, and is currently in review. The main methods, results and conclusions are below.)

Optimal partitioning theory, which states that plants should allocate biomass to the organ that acquires the most limiting nutrient, has been used to explain variation in biomass allocation for several decades. However, more recent studies have put the

generality of Optimal Partitioning Theory (OPT) into question by showing that much of the variation attributed to OPT can be explained by changes in size. In addition, plants can compensate with changes in environmental conditions by changing other factors, such as leaf morphology and physiology. We combine allometric biomass partitioning with OPT by examining the variation in biomass allocation after the effects of size are considered for plants grown at two different nutrient levels.

This study examines how two different nutrient levels affect variability in biomass allocation, after accounting for size. An extra sum-of-squares test was used to determine how much treatment contributed, to the variation in partitioning not accounted for by size. The directionality of nutrient level on partitioning patterns was used to determine if patterns predicted by OPT occurred intra and interspecifically for 48 species that varied in evolutionary history and growth form. Plants allocating biomass according to OPT should allocate more biomass to leaves and stem mass at the expense of root mass, for a given body size, at higher nutrient levels. An allometric effect was also examined using a simple t-test. Plants in the higher nutrient level were predicted to be larger in size.

The majority of the species, 85%, showed at least one pattern of biomass partitioning that was predicted based on OPT. Additionally, 70% of the species showed an increase in size at higher nutrient levels. Although many of the species followed OPT, the manner in which they showed OPT patterns varied. Some species demonstrated an increase in stem and leaf mass at the expense of root mass, others increased only stem or

leaf mass at the expense of root mass, and some species only altered partitioning to one organ. This variation in response led to a smaller interspecific effect of nutrient level.

The majority of plants appear to demonstrated both a plastic OPT response to an increase in nutrients in addition to an allometric response. These results indicate that allometric and partitioning response to environmental differences can be combined to develop a more inclusive understanding of how plants should allocate biomass in response to environmental variation. Additionally, patterns of partitioning, caused by alternate strategies, may vary between species, lead to a decrease in large-scale interspecific patterns.

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APPENDIX A

ORGAN PARTITIONING AND DISTRIBUTION ACROSS THE SEED PLANTS:
ASSESSING THE RELATIVE IMPORTANCE OF PHYLOGENY AND FUNCTION

Megan C. McCarthy, Brian J. Enquist and Andrew J. Kerkhoff

Abstract

Understanding how plant biomass is distributed between roots, stems and leaves is central to many questions in life history evolution, ecology, and ecosystem studies. Current ecological dogma states that patterns of biomass partitioning result from environmental differences. However, there are methodological issues associated the role of plant size. In addition, the importance of evolutionary history in biomass distribution is unclear. Here, we assess the relative importance of evolutionary history and growth form on how biomass, not accounted for by plant size, is partitioned between plant organs. Our analyses indicate that while growth form was significantly correlated with variation in biomass distribution and partitioning, phylogeny appears to be the strongest factor. Variation in biomass distribution is phylogenetically conserved for leaf mass, but not for stems, roots and annual production, suggesting that these factors may be more plastic. Leaf mass was the only organ with a considerable portion of the residual variation from growth form, which appears to be largely the result of differences in leaf traits. Our results have important implications for ecological studies; such that partitioning studies must first assess the role of plant size *and* evolutionary history in order to fully understand variability in biomass partitioning and distribution.

Key words: plant allometry, residual variation, phylogenetic conservatism, specific leaf area, growth form

Introduction:

Seed plants display large diversity in both form and function. An understanding of the factors that control how plants partition metabolic production has been sought for over a century (Kny 1889) and remains an important focus in many areas of ecology and evolutionary theory (Iwasa 2000). Many studies have emphasized that within and across plant species there is much variability in the partitioning and distribution of biomass between organs. This variation has been largely attributed to factors such as environmental disparity (Davidson 1969; Hunt and Burnett 1973; Bloom et al. 1985; Hunt and Nichols 1986; Iwasa 2000) and growth form (Monk 1966; Mooney 1972, Tilman 1988). A majority of these studies have focused on optimal partitioning theory, which postulates that plants should allocate biomass to the organ that acquires the most limiting resource (Davidson 1969; Hunt and Burnett 1973; Hunt and Nichols 1986; Iwasa 2000).

The terms biomass partitioning and biomass allocation are used interchangeably in the literature. For the purposes of this paper, both partitioning and allocation will refer to the residual variation in biomass being added annually to any particular organ from the production available. We will use the term distribution to refer to residual variation in the total amount of biomass in stems, roots and leaves relative to the total mass for a given plant (Reich 2002). It is important to point out that distribution is explained by both accumulated allocation of annual production and accumulated loss from various factors (Reich 2002), implying that plasticity to environmental heterogeneity may have minimal effect. However, it can also be argued that plant species or different populations

should demonstrate distribution patterns that follow optimal partitioning theory, such that plants adapted to particular environmental conditions, such as shade or drought, should demonstrate optimal distribution patterns according to where they live on the resource gradient (Chapin 1980, Givnish 1988, Tilman 1988). Though, instead of a plastic response to environmental variability, this may be both a plastic and adaptive response to the environment. Therefore, although biomass distribution is not identical to biomass partitioning, it is often used to understand partitioning when true allocation data is not available (Reich 2002).

There are a couple of potential problems with interpreting plant organ biomass partitioning in the light of optimal partitioning theory. First, recent studies have indicated variation in biomass partitioning and distribution may often reflect, at least in part, differences in plant size (Coleman et al. 1994; McConnaughay and Coleman 1999; Bernacchi et al. 2000; Enquist and Niklas 2002). Thus, variation in biomass partitioning and distribution may not reflect local plastic responses or even recent selective pressures but instead reflect differences in the sizes of plants being studied. Second, very little emphasis has been placed on the effect of phylogeny on patterns of organ biomass distribution and plasticity of partitioning. Evolutionary history appears to be important to several areas of plants form and function (Antunez et al. 2001; Brouat et al. 1998) and there is evidence of its importance to plasticity in biomass partitioning (Osone and Tateno 1995). So, it is unclear if current patterns of distribution may also largely reflect evolutionary history. Additionally, factors such as plant growth form (Monk 1966; Körner 1994) and leaf morphological and physiological traits (Osone and Tateno 1995)

may provide additional constraints on plant biomass distribution. Therefore, in order to understand the contributions of environmental variation to variation in biomass partitioning, it is important to also consider the roles of size, phylogeny, and functional differences.

Since the pioneering work of Pearsall (1927), several studies have shown that variation in plant biomass partitioning and distribution can often largely be related to size (Coleman et al. 1994; Bernacchi et al. 2000; Enquist and Niklas 2002). In an extension of metabolic scaling theory, Enquist and Niklas (2002) showed that seed plants, from small herbaceous seedlings to large conifers, appear to be following very similar rules for how their biomass is distributed. Based on constraints relating to the transport of resources from the ground through the plant and the photosynthetic harvesting capacity of the leaves, they predicted that: (i) leaf mass, M_L , should scale to the $3/4^{\text{th}}$ power of stem mass, M_S ; (ii) M_L should scale to the $3/4^{\text{th}}$ power of root mass, M_R ; (iii) and M_S should scale isometrically to M_R (see Enquist and Niklas 2002 for a complete description of the mechanistic constraints due to size). Or when written in the form of a power law:

$$M_L = \beta_1 M_S^{3/4} \quad (1)$$

$$M_L = \beta_2 M_R^{3/4} \quad (2)$$

$$M_S = \beta_3 M_R \quad (3)$$

where M_S , M_R , and M_L are stem, root, and leaf mass respectively and the Beta terms are normalization constants. The beta values reflect differences in biomass distribution not accounted for by size (further detail on the model in Enquist and Niklas 2002; Niklas and Enquist 2002; or metabolic scaling theory in general West et al. 1999). Extensive data for

conifers, monocots and eudicots spanning 6 orders of magnitude generally fit the predicted relationships. Plant size explained 97 to 99% of the variation in biomass distribution to leaf, stem, and root mass (Enquist and Niklas 2002). However, there existed about one order of magnitude residual variation around these allometric scaling relationships (Figure 1). Thus, while plant size alone explained most of the variation in organ mass distribution there still remains significant unexplained residual variation. In this paper we focus on some of the processes that may be driving this residual variation.

The seemingly great differences between the studies that show biomass partitioning to be extremely variable and studies that show biomass partitioning to be largely attributed to size are explained by the approaches of the studies. The first difference in the approach is the role of scale. Scaling studies look over enormous size ranges. As a result variation in biomass partitioning, once size is controlled for, i.e. residual variation in allometric plots, is usually much less than the variation explained by size (Figure 2a). In comparison, many studies may look across only half an order of magnitude variation in size, so most of the variation may appear to be related to other factors (Figure 2b). Secondly, many biomass partitioning studies have used root-to-shoot ratios to assess differences in allocation. However, as shown in Eqs. 1-3, organ ratios (i.e. the root to shoot ratio, M_L+M_S/M_R), must vary just from changes in plant size. This is because the exponent that governs the scaling of root and shoot mass is not isometric (i.e. 1). Therefore, an exponent less than one, which is the case for the global dataset, will lead to a decrease in the root-to-shoot ratio with increases in size. Interestingly, many optimal partitioning studies have found that root to shoot ratios decrease with

increases in water and nutrients (Davidson 1969). In short, in examining the role of environment and evolutionary history on biomass partitioning or distribution one must remove the influence of plant size.

Here we unify the perspectives used in biomass partitioning and distribution studies, by examining how phylogeny and growth form (i.e. evergreen trees, deciduous trees, shrubs, forbs and graminoids), as well as leaf trait differences that may largely explain leaf mass differences between Angiosperms and Gymnosperms, influence the residual variation in biomass partitioning and distribution, once plant size is controlled for. Specifically, we were able to address: *(i)* the relative importance of phylogeny versus growth form in residual biomass partitioning and distribution; *(ii)* whether biomass partitioning and distribution patterns in leaf, stem and root mass are phylogenetically conserved; and *(iii)* the role of leaf traits such as specific leaf area, photosynthetic rate and leaf lifespan in the distribution of leaf biomass.

Methods:

Data sets: A global database containing arboreal conifers, monocots and dicots, in addition to herbaceous species and seedlings, was used to examine the effects of phylogeny, growth form and leaf traits on biomass partitioning and distribution. The bulk of these data were from Cannell (1982), which contains organ mass and annual production rates for arboreal monocots, dicots and conifers, with additional data from the primary literature on organ biomass for seedlings and herbaceous species to broaden the size range (Enquist and Niklas 2002; Niklas and Enquist 2002).

The Cannell data (1982) are standardized to 1.0 hectare and contain plant density, total basal stem diameter, standing biomass and annual production rates of stem, bark, foliage, roots and fruits, as well as latitude, elevation and age of dominant species. Organ mass and production rates per individual were computed using total standing organ mass, total organ production, and plant density. Data used for analyzing biomass partitioning were generally from even-aged conspecific stands and production was often an average of several years, which would reduce variance in organ biomass and production. The full dataset contains over 200 species from around 600 sites world-wide.

Taxonomic information was added to the database from the division to the species level. Taxonomic classifications were used as a proxy for phylogenetic relatedness in some analyses. This approach appears reasonable since all of the taxa used are considered monophyletic by recent molecular analyses. Taxonomic information was obtained from the Angiosperm Phylogeny website (Stevens 2001 onwards) for angiosperms, which bases phylogenies on the most recent molecular analyses of angiosperms with nodes having substantial support, greater than 80% bootstrap values. Phylogenetic information for Gymnosperms was obtained from the national plants database (USDA 2002). In addition, these analyses and their results were backed up with phylogenetic analyses based on node ages instead of taxonomic levels (see analyses).

An additional dataset was used to examine the relationship between leaf traits and biomass distribution. GLOPNET is a database containing multiple leaf traits such as leaf mass area (g cm^{-2}), maximum photosynthetic rate, leaf lifespan, and dark respiration rate

(Wright et al. 2004). Species in this dataset that overlapped with the biomass distribution dataset were used in the analyses.

Statistical analyses:

Residual Variation:

Residual variation from the fitted allometric scaling relationships was used in all of our analyses. The residuals were saved from the biomass distribution and allocation regressions for leaf, stem and root mass and production as well as total annual production. The regressions for leaf, stem and root mass were performed against total mass minus the response variable to create independence, while the regressions for annual production were performed against the total mass. The residuals for all of the biomass partitioning and distribution data are completely independent of size and therefore all further analysis are looking at the variation in partitioning and distribution after the effects of size are considered.

Phylogenetically Structured Variation:

This analysis was adapted from an analysis examining phylogenetically structured environmental variation (Desdevises et al. 2003). A series of multiple regressions were used to determine the amount of residual variation in biomass and production explained by phylogeny, growth form, and both phylogeny and growth form. The variation due to phylogeny is measured using a patristic distance matrix of distances, a matrix of distances between all of the tips in the phylogeny, which was produced in phylocom

(<http://www.phylodiversity.net/phylocom/>; Webb et al. 2004). The node ages are determined from the fossil record and the phylogenetic relationships are determined using recent molecular analyses (Stevens 2001 onwards). Given that phylogenetic relationships are based on molecular analyses, the risk of homoplasy should be reduced. In addition, simulations suggest that error in branch lengths and the tree structure should have little effect on phylogenetic analyses (Martins and Garland 1991; Diaz-Uriarte and Garland 1996).

The mean residuals for stem, root and leaf mass and production were used for each species. The first multiple regression looks at the effects of growth form on the trait of interest (in this case, stem, root and leaf mass and production residuals). The next multiple regression assesses the effect of phylogeny on the trait of interest. The variation due to phylogeny is determined with a phylogenetic distance matrix produced in phylocom, which is then put into a Principle Components Analysis to determine the axes of variation. The multiple regression then assesses the effect of all the principle components (PCs) on the trait of interest. The third multiple regression looks at the effects of growth form and phylogeny, using the phylogenetic PCs that had a significant effect from the previous analysis, on biomass residuals. The variation because of growth form is equal to the R^2 from the 3rd analysis minus the R^2 from the 2nd. The variation from phylogeny is equal to the R^2 from the 3rd analysis minus the R^2 from the 1st. And the variation from both is equal to (the R^2 from the 1st + the R^2 from the 2nd) – the R^2 from the 3rd. The residual variation, not accounted for by phylogeny or growth form is

equal to 1 - the sum of the previous 3 computations (Desdevises et al. 2003 for further details).

Phylogenetic Trait Conservatism:

Two separate analyses were used to examine trait conservatism in biomass distribution and allocation. The first method was a nested analysis of variance performed in R. The nested ANOVA partitions variance into each taxonomic level, where variation at each level subtracts out variation at the next higher level. Taxa used in these analyses were all considered monophyletic (Stevens 2001 onwards). Traits in which a large amount of the variation is contained within higher taxonomic levels indicate that the trait is phylogenetically conserved (Promislow et al. 1992; Niklas 1994; Nealen and Ricklefs 2001; Guo et al. 2003). The nested ANOVA was performed on leaf, stem, root mass and production residual variation using the nested taxonomic levels as the explanatory variables.

An additional analysis was used to determine trait conservatism to correct for taxonomic levels comparing clades of different ages, which is a shortcoming of using taxonomy as a proxy for phylogeny (Martins and Garland 1991). The program Phylocom (Webb et al. 2004) was used to determine which traits were phylogenetically conserved. The measure of trait conservatism is essentially determined by examining the magnitude of contrasts across the tree (<http://www.phylodiversity.net/phylocom/>; Webb et al. 2004). If most of the divergences between related species have similar trait values, then the phylogenetic signal is stronger and indicates a more conserved trait. A nonconserved

trait should have large trait differences near the tips of the tree and smaller trait differences in the vicinity of the root. The phylogenetic megatree used in this analysis (Figure 3) was generated using phylomatic (<http://www.phylodiversity.net/phylomatic/>; see also Webb et al. 2004), with branch lengths added in Phylocom. Phylomatic uses the Angiosperm Phylogeny Website as the backbone of its phylogenies, which uses recent molecular phylogenetic analyses and nodes with >80% Bootstrap support (Stevens 2001 onwards). The use of recent molecular data should reduce the risk of homoplasy. In addition, it is important to note that error in branch lengths and minor error in phylogenetic relationships appears to have negligible effects on phylogenetic analyses (Martins and Garland 1991; Diaz-Uriarte and Garland 1996). Significance testing for trait conservatism is based on 1000 randomization of the trait values across the tips. The average trait residuals for each species were used in the analyses.

Angiosperm/Gymnosperm Divergence:

Based on the results of the trait conservatism and phylogenetically structured variation analyses, variation between angiosperms and gymnosperms were investigated. Differences were examined for leaf mass, leaf annual production, total annual production, and production per unit leaf mass using an ANOVA on the mean residuals for each species.

Leaf Trait Analyses:

Angiosperms and Gymnosperms diverge in a number of respects. In addition to these clades having different evolutionary histories, they possess dissimilar leaf morphologies and experience differing ecological habitats (Givnish 2002). Given our dataset consists of primarily evergreen conifers and deciduous Angiosperms that experience similar environmental conditions, the most notable difference between these clades in our dataset is their leaf morphology. We examined the relationship between leaf mass distribution and leaf traits to determine whether leaf traits may contribute to the divergence between Angiosperms and Gymnosperms. This was done by pairing the matching species in the GLOPNET dataset (Wright et al. 2004) and the biomass dataset. There were multiple measures for each measurement of each species, therefore the mean leaf mass residuals and mean leaf trait data were used. The \log_{10} of specific leaf area (SLA), maximum photosynthetic rate (A_{\max}), leaf lifespan (LL), as well as the first principal component axis from these three highly correlated traits (Reich et al. 1997; Wright et al. 2004) were used to explain variation in \log_{10} leaf mass residuals using an Ordinary Least Squares regression. An OLS regression was used because leaf traits and leaf mass residuals have different units of measurement and error is expected to be dissimilar (Sokal and Rohlf 1995). Specific leaf area and associated traits can vary within a species or even an individual depending on the microenvironment, therefore using the average for a species adds error to the analysis. Additionally, the individuals from the datasets and their locations were different. Therefore, any presence of a

relationship between leaf mass and leaf traits may signify a much stronger relationship than what actually appears in the analyses.

Predictions were made for the relationship between leaf mass residuals and leaf traits based on the assumption that leaf mass residuals should scale isometrically with leaf lifespan. A plant with leaves having a lifespan of two years should have twice as much leaf mass as a plant with a leaf lifespan of one year, all else being equal. Further predictions were easily made based on the relationships between leaf lifespan and the other leaf traits. Leaf lifespan is negatively related to the specific leaf area with an exponent of -1.34, and negatively related to the photosynthetic potential of the leaf, A_{\max} , with an exponent of -1.71 (Wright et al. 2004). Based on the prediction that leaf mass residuals should scale isometrically with leaf lifespan and the relationships between leaf traits, the following predictions were made between leaf mass residuals and leaf traits:

$$\text{Leaf mass residuals} \propto LL^{1.00} \quad (4)$$

$$\text{Leaf mass residuals} \propto A_{\max}^{-1.34} \quad (5)$$

$$\text{Leaf mass residuals} \propto SLA^{-1.71} \quad (6)$$

where LL is leaf lifespan, A_{\max} is the maximum photosynthetic rate and SLA is specific leaf area.

Results:

Phylogenetically Structured Variation:

A series of multiple regressions were used to determine how much of the residual variation in biomass distribution was the result of phylogeny, growth form, and both phylogeny and growth form. This analysis was performed for only stem mass, leaf mass and leaf production residual variation (Table 1). Both growth form and phylogeny were significant for stem mass ($R^2 = 0.11$, $p=0.0298$ and $R^2 = 0.53$, $p<0.0001$ respectively), leaf mass ($R^2 = 0.23$, $p=0.0002$ and $R^2 = 0.44$, $p<0.0001$ respectively), and leaf production ($R^2 = 0.13$, $p=0.0326$ and $R^2 = 0.31$, $p=0.0092$ respectively). The analysis was not used on root mass and stem, root and total production residuals because growth form was not significant. However, it is important to note that phylogeny was significant for root mass ($R^2 = 0.38$, $p<0.0001$), total annual production ($R^2 = 0.26$, $p = 0.0436$), and there was a trend for root mass production ($R^2 = 0.21$, $p = 0.0922$).

For stem mass residuals, most of the variation was the result of phylogeny. Only 1.6% of the variation was related strictly to growth form, 44% was from phylogeny and 8.9% of the variation in stem mass residual variation was because of both phylogeny and growth form. Almost half of the residual variation, 45.5%, was left to be explained by other factors. For leaf mass residuals, the largest fraction of variation was still explained by phylogeny. However, growth form explained a larger amount of the variation in leaf mass residuals, 12.9%, than it had for stem mass, 1.6%. Phylogeny explained 34.5% of leaf mass residual variation and 9.8% of the variation was explained by both growth form and phylogeny. A large fraction of the variation, 42.8%, was residual. Interestingly, leaf

mass production residuals varied from the leaf mass residuals in that very little variation was related to growth form, 0.4%, and the variation due to phylogeny, 18.1%, was similar to that due to both phylogeny and growth form, 12.9%. Most of the variation was residual, 69.6% (Table 1).

Phylogenetic Trait Conservatism:

A nested analysis of variance was used to determine whether biomass distribution and allocation is phylogenetically conserved for seed plants. Most of the variation in leaf, stem and root mass residuals was explained by the lower taxonomic levels (Figure 4a). However for leaf mass residuals, almost one-fourth of the variation was explained by the Division level, or the split between the angiosperms and gymnosperms, indicating that leaf mass is more conserved. Conversely, most of the variation in stem and root mass was at the species level or below, with no variation explained above the family level. While none of the annual production residuals were explained by the Division level (Figure 4b), a small amount of leaf mass production was explained by Class, and some stem and root mass production was explained by Order. However, the majority of leaf, stem and root production was explained by the species level or below.

A separate program, phylocom, was used as a second, more robust measure of trait conservatism. This analysis works similarly to the taxonomic nested anova, except instead of using taxonomic level to determine the spread of variation, node ages are used. Leaf mass residuals were significantly conserved ($p = 0.004$), however stem and root mass residuals in addition to leaf, stem, root and total annual production were not ($p =$

0.36, $p = 0.86$, $p = 0.64$, $p = 0.30$, $p = 0.35$ and $p = 0.29$ respectively), lending support to what was found by the nested ANOVA.

Angiosperm/Gymnosperm Divergence:

The considerable divergence in leaf mass between angiosperms and gymnosperms in addition to variation in leaf mass having a large component explained by growth form and phylogenetically structured growth form compels further investigation. Differences between angiosperms and gymnosperms were investigated by performing a t-test on the residual variation of leaf mass. Gymnosperms have higher leaf mass residuals than angiosperms ($p < 0.0001$, $R^2 = 0.49$). Despite the higher leaf mass residuals of gymnosperms, angiosperms had higher residuals in annual leaf production ($p = 0.0002$, $R^2 = 0.07$). Similarly, angiosperms have higher residuals for total annual production than gymnosperms ($p = 0.0188$, $R^2 = 0.03$). The combination of these factors lead to angiosperms having a much higher amount of production for a given leaf mass ($p < 0.0001$, $R^2 = 0.61$).

Leaf Trait Analyses:

Specific leaf area (SLA), leaf lifespan (LL), and maximum photosynthetic rate (A_{\max}) were compared with leaf mass residuals to determine whether these factors explain the differences between angiosperms and gymnosperms. The predictions based on the assumed relationship between variation in leaf mass and leaf lifespan, and the relationships among the leaf traits were:

$$\text{Leaf mass residuals} \propto LL^{1.00}$$

$$\text{Leaf mass residuals} \propto A_{\max}^{-1.34}$$

$$\text{Leaf mass residuals} \propto SLA^{-1.71}$$

where LL is leaf lifespan, A_{\max} is the maximum photosynthetic rate and SLA is specific leaf area. Leaf lifespan was positively related to the leaf mass residuals with the confidence interval of the slope including the prediction of one (Figure 5a) ($\beta = 0.84$; 95% CI: 0.48, 1.20; $R^2 = 0.45$; $p < 0.0001$). The maximum photosynthetic rate and SLA were both negatively related to the leaf mass residuals (Figure 5b, c). The relationship between A_{\max} and leaf mass residuals included the predicted exponent of -1.34 ($\beta = -1.49$; 95% CI: -0.91, -2.06; $R^2 = 0.51$; $p < 0.0001$). The relationship between SLA and leaf mass residuals was less than the prediction of -1.71, but followed the predicted direction ($\beta = -0.82$; 95% CI: -0.19, -1.46; $R^2 = 0.12$; $p = 0.0125$). Leaf mass residuals were also compared to the first principle component from a PCA of the leaf traits since leaf lifespan, maximum photosynthetic rate and SLA are highly correlated. The first PC of the three leaf traits was also negatively related to the leaf mass residuals ($\beta = -0.83$; 95% CI: -0.55, -1.11; $R^2 = 0.63$; $p < 0.0001$). This matches what was found with the previous regressions, such that plants with higher leaf lifespans, lower SLAs and A_{\max} values falling out higher in the leaf mass residuals and plants with lower leaf lifespans, but higher SLAs and A_{\max} values having lower leaf mass residuals (Figure 5d).

Discussion:

There have been numerous studies examining the effects of different ecological and evolutionary factors on plant biomass allocation and distribution (Monk 1966; Davidson 1969; Hunt and Burnett 1973; Bloom et al. 1985; Hunt and Nichols 1986; Tilman 1988; Iwasa 2000). However, recent studies have shown that some of the variation in biomass allocation can be attributed to changes in size (McConnaughay and Coleman 1999; Bernacchi et al. 2000; Enquist and Niklas 2002). Based on the constraints of transporting resources and the photosynthetic harvesting capacity of leaves, an allometric analysis made several predictions concerning how much biomass should be distributed to a particular component relative to other components (Enquist and Niklas 2002). These predictions were supported by the empirical evidence with 97 – 99% of the variation in biomass distribution being explained by size with about one order of magnitude residual variation. These allometric models and the variation surrounding them provide the basis to understand how other evolutionary and ecological factors affect biomass distribution.

Phylogenetically Structured variation

Phylogeny has been shown to be important to several areas of plant form and function (Antunez et al. 2001; Brouat et al. 1998), however, most biomass distribution and partitioning studies have failed to take evolutionary history into consideration. We attempt to determine how much of the variation in biomass allocation and distribution is constrained by phylogeny. Because the two dominant clades in our study differ both morphologically and phylogenetically, we first try to partition out the variation due to

phylogeny from that due to growth form, specifically the evergreen versus the deciduous habit.

While many studies have overlooked the effects of phylogeny on biomass allocation and distribution, our results indicate that phylogeny alone can explain a significant fraction of biomass partitioning and distribution not explained by plant size. Importantly, phylogeny explains more of the residual variation in stem mass, leaf mass and leaf production than growth form and growth form related to phylogeny (Table 1). In addition, growth form was not even a significant factor in root mass and production variation and total annual production variation, unlike phylogeny. The combination of phylogeny and growth form leave less than half of the variation in stem and leaf mass to be explain by other more proximate sources such as local environmental variation. Interestingly, a much larger portion of the variation was residual for leaf production than for leaf mass, suggesting that proximate factors, particularly environment, may be a much more important factor to variability in biomass allocation to leaves than leaf mass distribution. Additionally, neither phylogeny or growth form explained significant variation in stem annual production; again suggesting that biomass allocation to stems is more plastic than stem mass distribution. The combination of these results suggests that while there is some phylogenetic constraint on biomass partitioning, there is still a large portion available to plastic responses to environmental variability. Additionally, the distribution of biomass, which incorporates both allocation and loss over time, is further constrained by evolutionary history.

Phylogenetic Trait Conservatism

In the nested ANOVA, the location of taxonomic variation in biomass distribution and allocation is used to indicate the conservativeness of the trait being examined (Niklas 1994). If most of the variation in the trait is contained in the higher taxonomic levels, this indicates that the trait is conserved, while variation in the species or residual level indicates more plasticity in that trait. Our analysis showed that about 30% of the variation in leaf mass is explained above the family level, with 20% of the variation explained by the divergence between angiosperms and gymnosperms (Figure 4a). Conversely, neither stem nor root mass had any variation explained above the family level. While all three organ masses had significant fractions of variation explained at the lowest taxonomic levels, suggesting considerable plasticity, the large fraction of leaf mass variation explained by the division level also demonstrates a reasonable amount of phylogenetic conservatism. In addition, previous analyses have shown a strong tradeoff between stem and root mass residuals existing even within species (McCarthy et al. in prep.), further indicating that these traits may be more plastic to adjust to microenvironmental variation. While leaf, stem and root production had a small amount of variation explained above the family level, these traits contained most of the variation, 60-83%, at the lowest taxonomic levels (Figure 4b), with no variation explained by Division. A small amount of variation in leaf annual production was within Class, however, more than 50% of leaf production variation was contained in the species level and about a third of the variation in leaf production was residual. The production results suggest that

biomass partitioning may demonstrate considerable plasticity. Additionally leaf partitioning appears to be much more plastic than leaf mass distribution.

This analysis was supported by an additional analysis in phylocom. Phylocom (Webb et al. 2004) bases the measure of trait conservatism on the amount of variation explained by older nodes in the phylogeny compared to younger nodes instead of different taxonomic levels. Not surprisingly, the results from Phylocom showed that leaf mass residuals were significantly phylogenetically conserved ($p = 0.004$). However, stem and root mass were not ($p = 0.36$ and 0.86 respectively). Variation in leaf, stem, root and total annual production was also not phylogenetically conserved ($p = 0.64$, $p = 0.30$, $p = 0.35$ and $p = 0.29$ respectively).

Angiosperm/ Gymnosperm Divergence:

The large amount of variation in leaf mass residuals explained by the angiosperm/gymnosperm divergence was followed up by examining the difference in residuals for leaf mass and production between these clades. Gymnosperms had greater amounts of leaf mass for a given body size than angiosperms. However, this greater amount of leaf mass was not because gymnosperms produce more leaf mass each year. Angiosperms have higher annual leaf production, indicating that the higher leaf mass that gymnosperms have results from retaining leaf mass from previous years instead of adding more leaf mass per year. It may also be expected that because of the higher amounts of leaf mass, gymnosperms should be able to achieve higher total annual production rates. This also does not occur. Although gymnosperms have more leaf mass to

photosynthesize with, angiosperms have higher total yearly production. These differences in leaf mass and production between angiosperms and gymnosperms lead to angiosperms having an order of magnitude higher production for a given leaf mass.

Because angiosperms and gymnosperms differ in many ways besides than just organ partitioning, it is difficult to determine whether the divergence in leaf mass is the result of phylogeny or general growth form and functional differences. Angiosperms and gymnosperms possess diverse leaf morphologies and physiologies, occupy dissimilar ecological niches in addition to having different evolutionary histories (Midgley 1991; Givnish 2002). While the bulk of the variation in leaf, stem and root mass appear to be the result of phylogeny, leaf mass is the only factor that also has a substantial portion of the variation because of growth form. In fact, almost a quarter of the variation in leaf mass can be attributed to growth form and phylogenetically structured growth form, suggesting the differences in leaf morphology between these clades may pose additional constraints to the evolutionary history.

Leaf Trait Analyses:

Work on leaf traits by Reich et al. (1997) provides an understanding of how gymnosperms can have greater leaf mass while adding less leaf mass per year, and how angiosperms can have higher annual production with less leaf mass. Specific leaf area (SLA), which is leaf area divided by leaf mass, is positively correlated with maximum photosynthetic rates. In addition, SLA is negatively correlated with leaf life span (Wright

et al. 2004). A leaf with smaller SLA will therefore have lower photosynthetic rates for a given leaf mass and a longer lifespan than a leaf with higher SLA. Because gymnosperms tend to have lower SLAs than angiosperms, these patterns would lead to gymnosperms having higher leaf masses, because of accumulation of long lived leaves, and lower production rates per unit leaf mass.

The relationships between leaf mass and leaf traits can be incorporated into the allometric coefficient of the leaf mass allometric regression such that $M_L = \beta M_T^{3/4}$ where $\beta = LL^{-1}$. This demonstrates that variation in leaf mass is a function of the lifespan of leaves and therefore the number of cohorts of leaves contained at any one time. Several predictions were made based on this assumption and the relationships between the leaf traits.

We tested the predictions by comparing the specific leaf area, leaf lifespan and maximum photosynthetic rate to the leaf mass residuals. The relationship between leaf lifespan and the leaf mass residuals was close to the prediction of unity, 0.84, with the confidence intervals including one, 0.49 – 1.20 (Figure 5a). The R^2 was relatively high for this relationship, 0.45, with leaf lifespan accounting for almost half of the variation in leaf mass residuals. Similarly, the relationship between the potential photosynthetic rate and leaf mass residuals (Figure 5b) was very close to the prediction of -1.34 (*slope* = -1.49; 95%CI -0.91, -2.06), with potential photosynthetic rate explaining slightly more than half of the variation in leaf mass residuals ($R^2 = 0.51$). The slope of the relationship for specific leaf area and leaf mass residuals (Figure 5c) was greater than the prediction of -1.71 (*slope* = -0.82; -0.19, -1.46) with a much lower R^2 value ($R^2 = 0.12$). The lack of

fit for this relationship is not surprising. First of all, the measurements for the leaf traits were not from the same individuals as the measurements the leaf mass was taken from. Because SLA can vary from individual to individual depending on the microenvironment, this is most likely adding large amounts of error to these analyses. Additionally, the relationship between SLA and leaf lifespan, which was used to generate the predicted exponent of -1.71 between SLA and leaf mass residuals, contains much more variation than the other relationships between leaf traits because it is affected by precipitation (Reich et al. 1997; Wright et al. 2004). This extra variation leads to a lower R^2 value, leading to an increase in the RMA slope, which is equal to the OLS slope divided by r (Sokal and Rohlf 1995). The least squares slope between SLA and leaf lifespan is -1.11, which is included in the confidence interval between SLA and the leaf mass residuals, -0.19, -1.46.

To further support the strength of the relationship between leaf mass residuals and the leaf traits, the first principle component axis of the leaf traits is highly correlated with leaf mass residuals (Figure 5d) ($slope = -0.83$; $R^2 = 0.63$; $p < 0.0001$). Plants with higher leaf lifespan, lower SLA and photosynthetic potential, such as conifers, fall out higher in the leaf mass residuals, as would be expected, and plants with lower leaf lifespans, higher SLA and photosynthetic potential, like many deciduous angiosperms, have lower leaf mass residuals. The strength of the relationships between leaf traits and leaf mass residuals is extremely impressive given the amount of error expected from pairing datasets with different individuals and using the mean leaf mass residuals and leaf traits. These relationships may largely explain the differences between angiosperms and

gymnosperms in their leaf mass distributions. Additionally, these patterns suggest that leaf morphology may constrain leaf mass distribution.

Conclusions:

It has become increasingly apparent that body size governs much of form and function for both animals (West et al. 1997) and plants (Niklas 1994; Enquist and Niklas 2002; Niklas and Enquist 2002). While the majority of biomass partitioning and distribution studies have concentrated on environmental effects (Davidson 1969; Hunt and Burnett 1973; Hunt and Nichols 1986; Iwasa 2000), most have ignored the importance of body size, phylogenetic and growth form constraints. The biomass partitioning patterns found by Enquist and Niklas (2002) were used as a baseline to understand how other factors may constrain biomass distribution and allocation after the effect of size is accounted for.

Our results indicate that phylogeny seems to explain a significant fraction of the variation in biomass partitioning when looking globally across large body size ranges. Also, angiosperms and gymnosperms show important differences in leaf mass and production rates that appear to be related to leaf morphology and physiology. Thus, by using the allometric models as a baseline to understand residual variation in biomass partitioning and distribution, in addition to incorporating differences due to leaf phenology and other factors into the allometric coefficients of the biomass distribution model, we can obtain a more detailed understanding of processes influencing variation in organ partitioning across the Spermatophytes. Our results have important implications

for ecological studies. Understanding interspecific differences in organ distribution must first assess constraints due to plant size, evolutionary history and growth form.

Furthermore, plant biomass partitioning studies need to consider constraints due to allometry and perhaps growth form. For example, a species that displays limited variation in leaf morphology may also be less plastic in biomass partitioning. We suspect that future studies that incorporate phylogeny, size, function, and environmental conditions together will likely provide insight into the ecological and evolutionary processes that shape differences in botanical biomass partitioning and distribution.

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Figure legends

Figure 1. Biomass allometric relationships between leaf, stem and root mass for conifers, monocots and dicots from a global compilation of seed plants. Predicted relationships are in the upper left hand corner and actual allometries are in the lower right hand corner of each graph. Graphs are from Enquist and Niklas 2002.

Figure 2. Allometric relationship between stem mass and total mass minus stem mass viewed across two different size ranges. A) Allometric relationship between stem mass and root plus leaf mass (or total mass minus stem mass). Residual variation, variation on the y-axis (arrow), around the relationship appears to be very small when examined over twenty orders of magnitude. The square is blown up in graph B. B) Blow up of the square in graph A. The residual variation in stem mass is similar to and even greater than the variation in body size. Therefore, it can appear that size does not play as important a role in biomass distribution when looked across small size ranges.

Figure 3. Phylogenetic supertree of seed plant species generated using phylocom. Branch lengths are based on fossil data and are not drawn to scale. The relationships in this tree are used in the phylogenetic analyses.

Figure 4. The amount of variation in distribution and partitioning explained by each taxonomic level. Fractions of variation for each taxonomic level of each component are all significant, mostly $p < 0.0001$, but all $p < 0.05$. A) Most of the variation in leaf, stem and root mass residuals is explained by the species and below the species (residual) level. Only leaf mass residuals have variation explained above the family level. B) The majority of the variation in leaf, stem, root and total annual production residuals is explained by the species and below the species (residual) level. A small portion of the variation in leaf, stem and root production is explained above the family level, however no variation is explained at the division level.

Figure 5. Relationships between the mean leaf mass residuals and leaf traits for seed plant species. A) Leaf mass residuals are positively related to leaf lifespan. The predicted slope, actual slope with confidence intervals and the R squared value are in the bottom right hand corner. The confidence intervals overlap with the predicted slope of 1. B) Variation in leaf mass is negatively correlated with maximum photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$). The predicted slope, actual slope with confidence intervals and the R squared value are in the bottom left hand corner. The confidence intervals overlap with the predicted slope of -1.34. C) There is a negative relationship between leaf mass residuals and SLA. The predicted slope based on the RMA leaf trait relationships and OLS leaf trait relationships, actual slope with confidence intervals and the R squared value are in the bottom left hand corner. The confidence interval does not overlap with the predicted slope of -1.71, however it does with slope of -1.11 based on the OLS relationship between SLA and LL. D) Variation in leaf mass is negatively related to the first principle components axis from a PCA of LL, Amax, and SLA. There is no predicted slope for this relationship, however the predicted direction is negative. The slope and R squared values are listed in the bottom left hand corner.

Tables

Table 1

Variation for Stem Mass, Leaf Mass and Leaf Annual Production due to Growth Form,
Phylogeny and Phylogenetically Structured Growth Form.

Characteristic	Phylogeny and Growth form			Residual
	Related to Growth form			
	Related to phylogeny			
Stem Mass	0.016	0.089	0.440	0.455
Leaf Mass	0.129	0.098	0.345	0.428
Leaf Production	0.004	0.129	0.181	0.686

Note. The phylogenetically structured growth form variation is the variation that is explained by both growth form and phylogeny. The residual is the variation that is not explained by either phylogeny or growth form.

Figures

Figure 1.

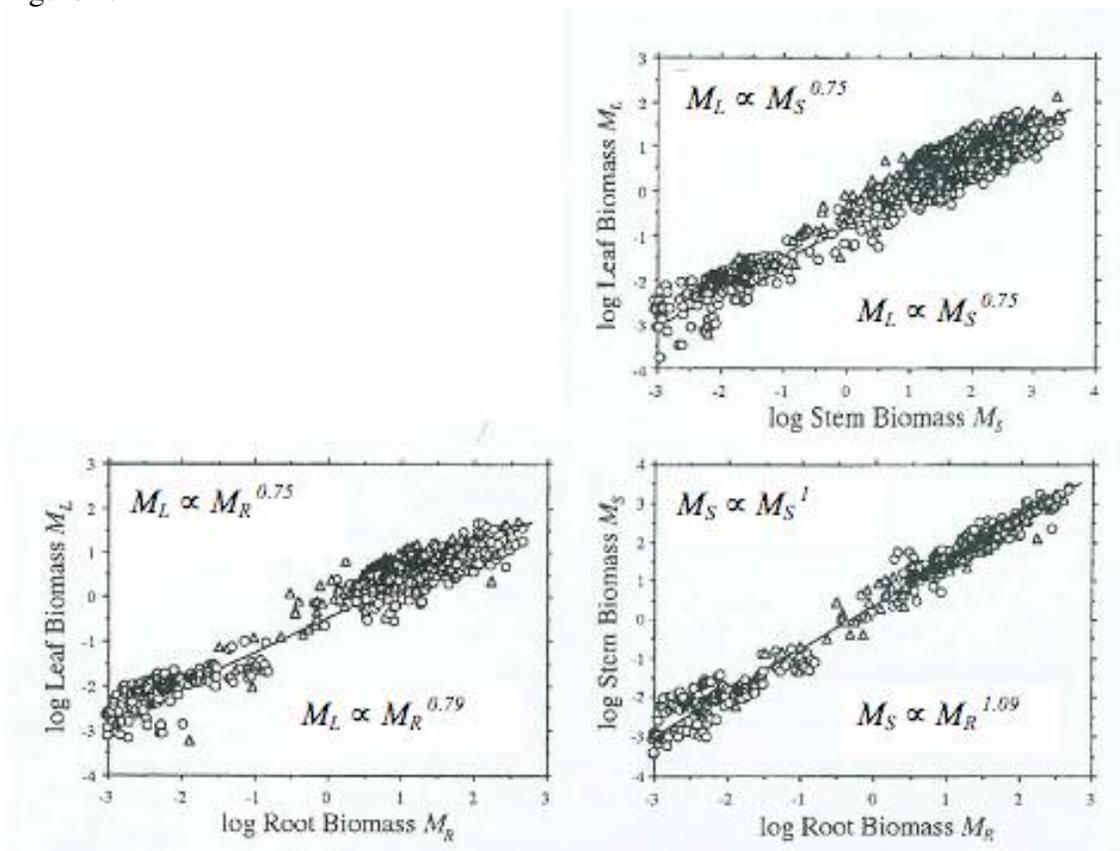


Figure 2a.

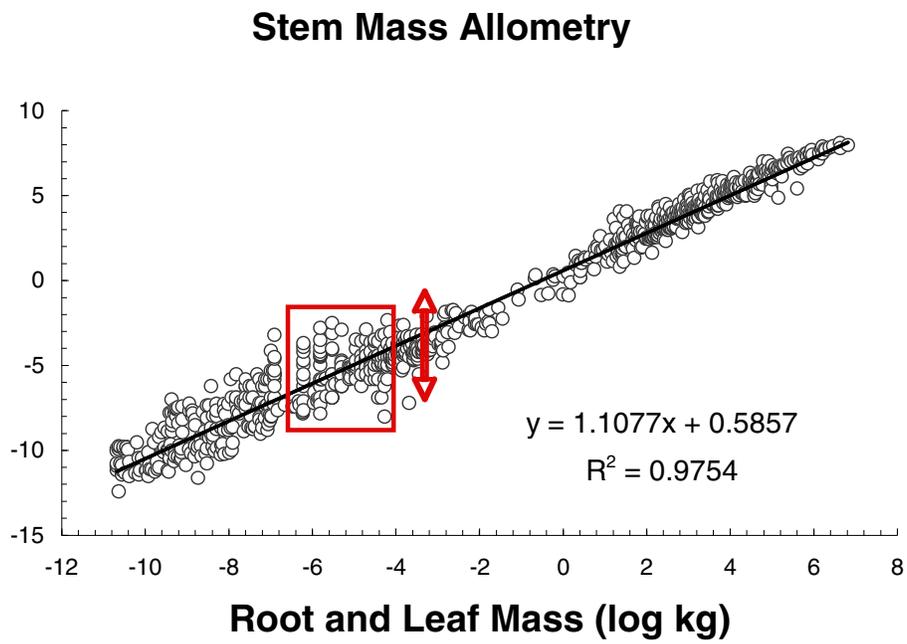


Figure 2b.

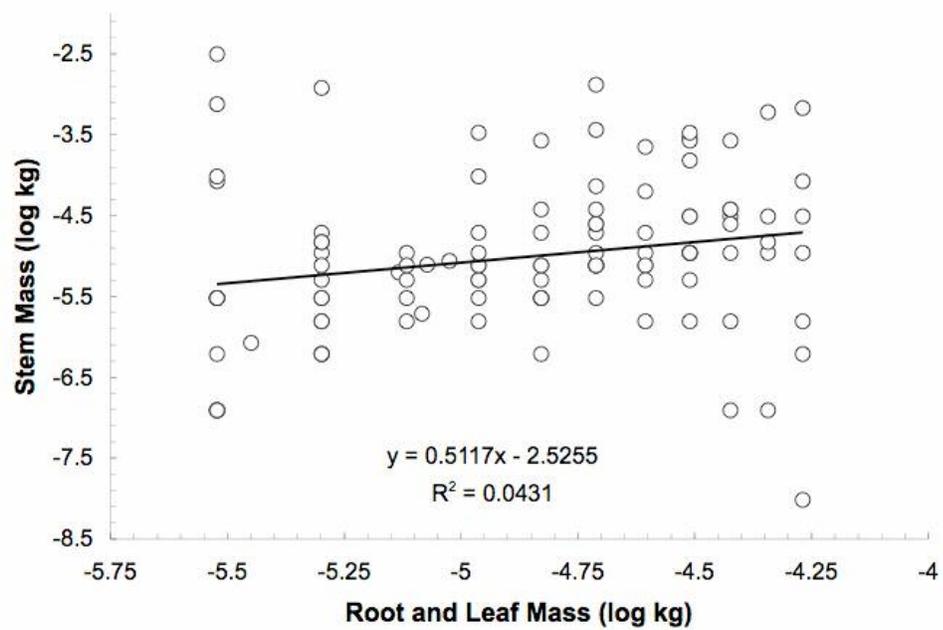


Figure 4a.

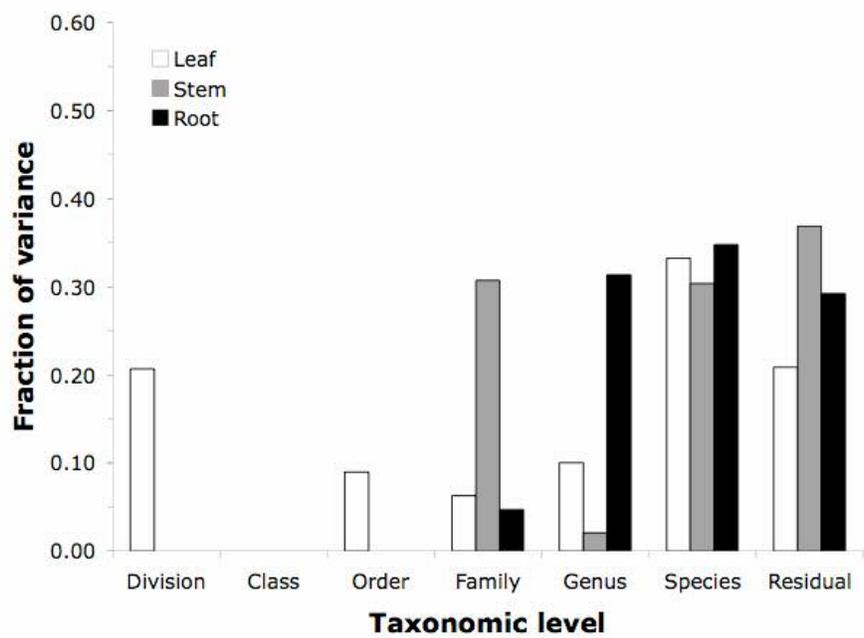


Figure 4b.

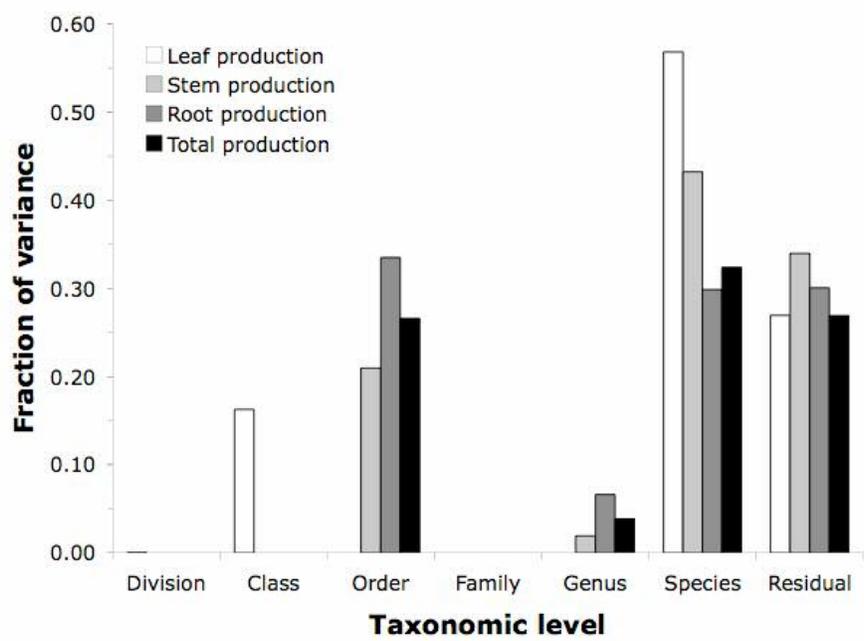


Figure 5a.

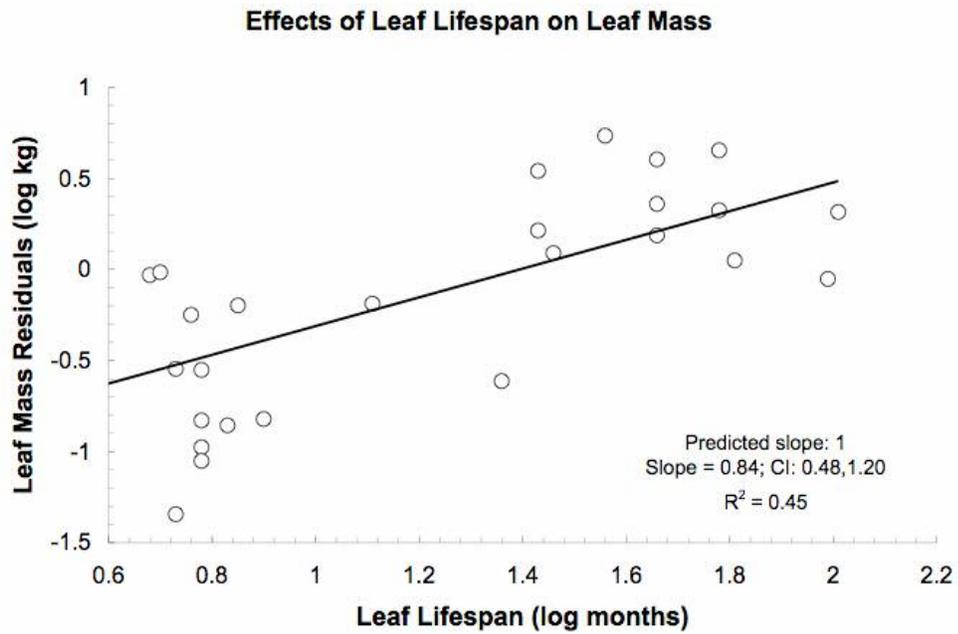


Figure 5b.

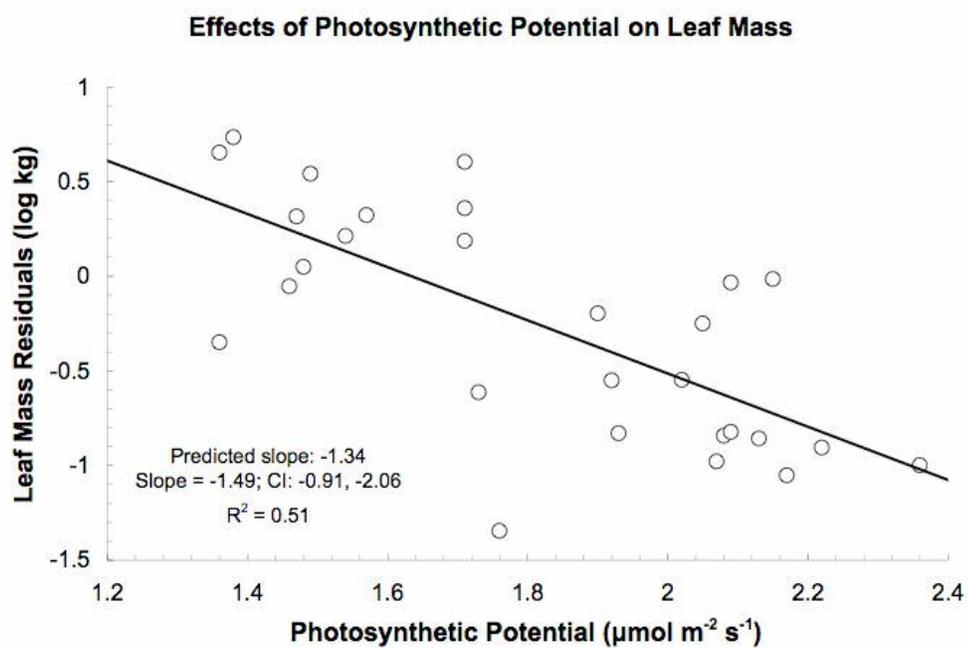


Figure 5c.

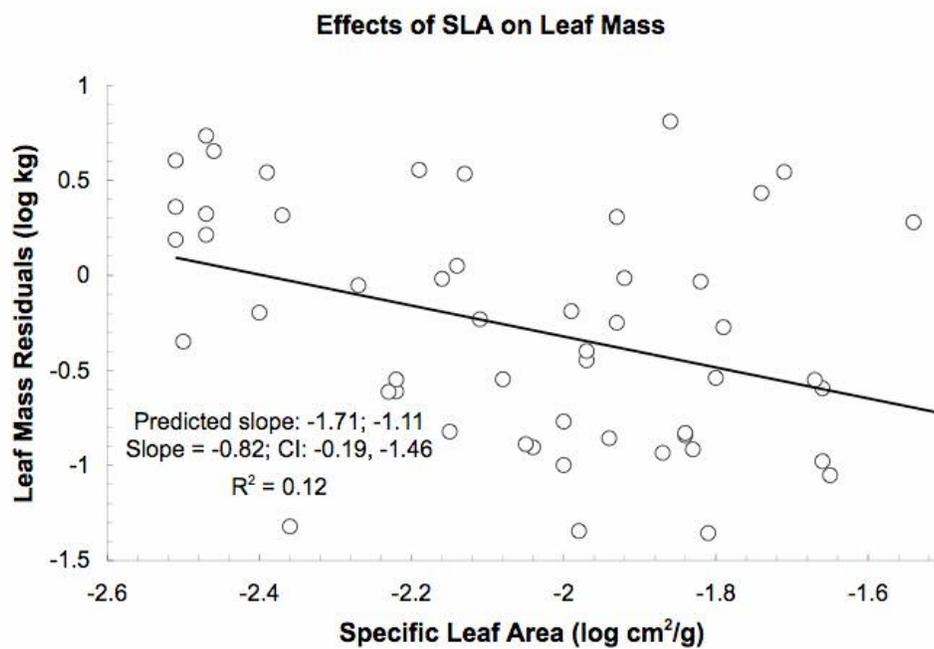
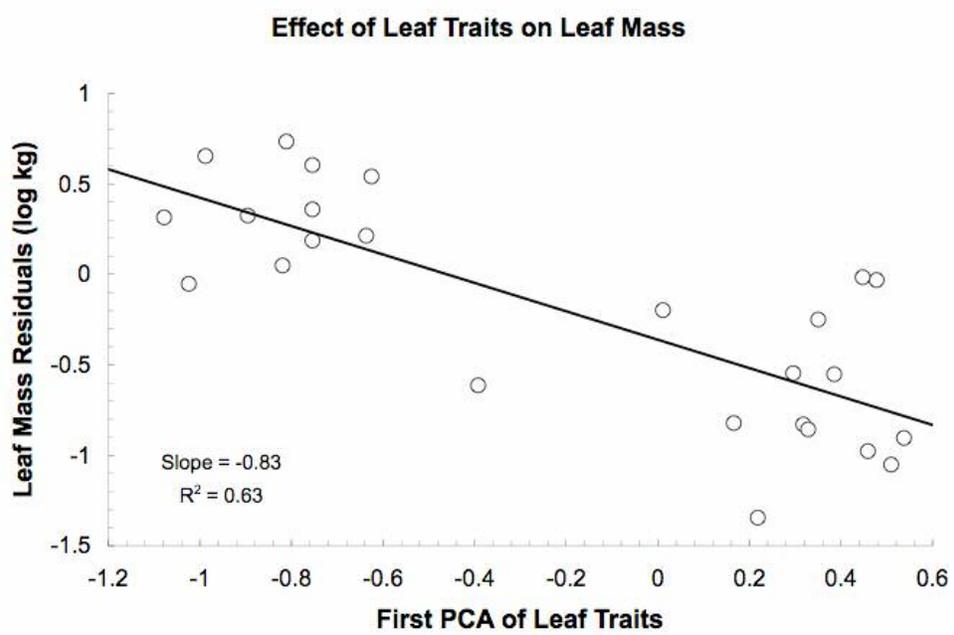


Figure 5d.



APPENDIX B

CONSISTENCY BETWEEN AN ALLOMETRIC APPROACH AND OPTIMAL
PARTITIONING THEORY IN GLOBAL PATTERNS OF PLANT BIOMASS
ALLOCATION

Megan C. McCarthy and Brian J. Enquist

Summary

1. Optimal Partitioning Theory (OPT) suggests that plants should allocate biomass to the organ that acquires the most limiting resource. An implied assumption of this is that there are tradeoffs in allocation between leaf, stem and root functions.
2. Recently, an alternative approach, Allometric biomass Partitioning Theory (APT), was developed to predict how plants should divide their metabolic production between leaves, stems and roots based on the constraints of body size. APT predicts that, for an allometrically ideal plant, leaf mass should scale to the $3/4$ th power of body size, and stem and root mass should scale isometrically to body size.
3. In this study we combine OPT with APT by investigating biomass partitioning not accounted for by allometric constraints across broad environmental gradients.
4. Intraspecific variability in biomass allocation shows correlations with environmental factors that would be predicted by OPT. However, interspecifically, these patterns do not appear or are greatly reduced.
5. Our study suggests that, after size is accounted for, intraspecific residual variation in biomass allocation may be partially explained by environmental factors in a manner consistent with OPT. However, the particular patterns vary between species, obscuring large-scale patterns.
6. In summary, differences due to environmental variability can be incorporated with allocation patterns related to total mass to help understand how plants should allocate biomass in response to both changes in size and environment.

Key words: biomass partitioning, environmental variation, optimal partitioning, plant allometry.

Introduction

Allocation of metabolic production in plants is a zero-sum dynamic. Differential allocation of production to leaf, stem and root mass must come at the expense of the other structures. Elucidating the forces that influence the allocation of metabolic production is a central focus in comparative plant ecology and evolution (Bazzaz and Grace 1997). The partitioning of metabolic production is the basis for much life-history (Iwasa 2000), and ecological theory interested in ecological dynamics and global change (Tilman 1988; Bernacchi et al. 2000). Several studies have emphasized the variability in biomass partitioning observed across plants. These studies primarily fall under Optimal Partitioning Theory (OPT), which suggests that plants should allocate biomass to the organ that acquires the most limiting resource (Thornley 1972; Bloom, Chapin and Mooney 1985). Optimal Partitioning Theory is a cornerstone of many theories in plant ecology and evolution (Grime 1979; Tilman 1988). However, the generality of OPT has lately been questioned (Coleman, McConnaughay and Ackerly 1994; Coleman and McConnaughay 1995; Müller, Schmid and Weiner 2000; Reich 2002). Specifically, much of the variation OPT claims to account for may in fact be driven by differences in plant size. Nevertheless, since some of these studies have tended to examine either the effects of size *or* of environmental variability, independent of plant size, it remains unclear whether there are general patterns in biomass partitioning in relation to environmental variation after size is considered.

The terms biomass partitioning and allocation are used interchangeably in the literature to mean both the dividing up of annual growth to stems, roots and leaves as

well as the standing amount of biomass in stems, roots and leaves at a particular point in time (Reich 2002). Our study specifically addresses the standing amount of biomass in stems, roots and leaves at a particular point in time. It is important to point out that this measure is accounting for both the accumulated partitioning of annual production and accumulated loss from various factors (Reich 2002). While many optimal partitioning studies address the division of new growth, it can also be argued that standing amounts of biomass in stems, roots and leaves should also follow OPT patterns due to the repeated optimally partitioned additions of new growth. In addition, it has also been reasoned that species or populations of plants adapted to particular environmental conditions, such as shade or drought, should demonstrate optimal partitioning patterns according to where they live on the resource gradient (Chapin 1980; Givnish 1988; Tilman 1988). Therefore, although these two measures of biomass allocation are not identical, both can and have been used to understand optimal partitioning when true allocation data is not available.

There is a large body of literature that generally supports predictions made by OPT. Plants have been shown to allocate more to roots when water or nutrients are limiting and more to shoots when light is limiting (Crist and Stout 1929; Davidson 1969; Hunt and Burnett 1973). Although numerous studies have supported optimal partitioning theory, several recent studies have shown that changes in plant size may lead to variation in biomass allocation when root-to-shoot ratios are used (Coleman et al. 1994; Coleman and McConnaughay 1995; Bernacchi et al. 2000; Müller et al. 2000; Reich 2002). The root-to-shoot ratio is expected to change with plant size if organ masses do not scale isometrically to each other. Multiple studies have shown that root and shoot mass often

do not scale isometrically (Müller et al. 2000; Enquist and Niklas 2002; Shipley and Meziane 2002; Robinson 2004). Therefore, the combination of altered resource supply, affecting growth rates, with non-isometric scaling of root and shoot mass can result in perceived changes in biomass partitioning with the use of root-to-shoot ratios.

Recently, an alternative biomass partitioning model, but not mutually exclusive to OPT, was developed to predict how plants should optimally divide their metabolic production between leaves, stems, and roots independent of the influence of the environment. The Allometric biomass Partitioning Theory (APT) model is based on allometric theory, which examines how organismal attributes change with body size according to the allometric equation: $\log Y_1 = \log \beta_0 + \alpha \log Y_2$, where β is the allometric constant, α is the scaling exponent, and Y_1 and Y_2 are interdependent variables for size (Peters 1983). This equation can also be written in the form of a power-law such that $Y_1 = \beta Y_2^\alpha$. APT predicts a series of scaling relationships that reflect how plants should partition biomass based on the constraints of maximizing photosynthetic harvesting capacity and resource transport, while minimizing hydrodynamic resistance and transport times (Enquist and Niklas 2002; Niklas and Enquist 2002). The allometric partitioning model predicts that leaf mass should scale to the 3/4th power of stem and root mass and stem mass should scale isometrically to root mass such that:

$$M_L = \left(\frac{1}{\beta_{13}} \right) M_R^{3/4} \quad (2)$$

$$M_L = \left(\frac{1}{\beta_{11}} \right) M_S^{3/4} \quad (3)$$

$$M_s = \left(\frac{\beta_{12}}{\beta_{13}} \right) M_R^1 \quad (4)$$

where M_s , M_R , and M_L , are stem, root, and leaf mass respectively. Note, the Beta terms are allometric constants that may vary across species or differing environments. (Enquist and Niklas 2002; Niklas and Enquist 2002).

Extensive data for conifers, monocots and dicots spanning 12 orders of magnitude fit the predicted relationships remarkably well (Enquist and Niklas 2002, Niklas and Enquist 2002). However, at any given whole-plant mass, there were one to two orders of magnitude variation in organ mass. This residual variation was hypothesized to reflect species-specific and potential ecological differences in allocation within and across species (Enquist and Niklas 2002; Niklas and Enquist 2002). Therefore, the residual variation in allometric partitioning in biomass should reflect Optimal Partitioning Theory.

A General Framework to Assess Optimal Partitioning Theory

While accounting for body size clarifies the discrepancy between optimal partitioning and allometric studies, it does not necessitate that optimal partitioning should be lacking after body size is accounted for. Intuitively it seems reasonable that plants should allocate more to roots if lack of water or nutrient availability is limiting their survival and/or reproduction. The partitioning relationships found by Enquist and Niklas (2002) provide an important theoretical baseline prediction by which to disentangle the effects of plant size and environmental variation. By using the variation not explained by size from the biomass partitioning patterns, we can assess how environmental conditions affect

allocation while controlling for allometric scaling of organ size. If the variation in organ mass distribution demonstrates optimal partitioning, then stem and leaf mass variation should be negatively related to the light availability and positively related to water availability while the root mass residuals should show the opposite pattern.

In this study, we use the Cannell database (Cannell 1982) to examine whether tradeoffs in biomass partitioning are related to broad-scale environmental differences both intra and interspecifically. However, attempting to address OPT interspecifically faces potential spurious phylogenetic effects. While relatively few studies have quantified phylogenetic constraints in plant biomass allocation, evolutionary history is important to many areas of plant form and function (Antunez, Retamosa and Villar 2001) and appears to be significant in biomass partitioning as well (Osone and Tateno 1995). Therefore, we address interspecific patterns in allocation both with and without correcting for phylogeny.

Methods

Data sets

A global database containing organ mass and annual production rates for arboreal conifers, monocots and dicots (Cannell 1982) was used to examine the effects of environmental variation on the residual variation in biomass allocation. The Cannell data (1982) is standardized to 1.0 hectare and contains plant density and standing biomass for stem, bark, foliage, and roots, as well as latitude and longitude. Individual biomass values were averages computed by dividing total stand organ mass by the number of

individuals in the plot. Data used for analyzing biomass partitioning were generally from even-aged conspecific stands.

There are several important details to be noted in the Cannell dataset. First of all, organ masses were computed using total standing mass and plant density and the individuals were generally from even-aged conspecific stands. Both of these factors reduce variance and therefore limit the potential of finding patterns. Despite these limitations, phylogenetic signals appear strongly in the organ mass residual variation (McCarthy et al. accepted IJPS). Furthermore, the majority of the individuals in the dataset are large trees, where a sizeable amount of biomass is structural. This contrasts with many OPT studies which concentrate on small individuals with very little structural support. However, OPT has been used to understand biomass partitioning in trees as well (Givnish 1988; Tilman 1988; Ingestad and Argen 1991; King et al. 1999). In addition, the woody stem and root mass that makes up a large amount of biomass in trees, is important for both light and resource acquisitions, as well as for several other functions (Körner 1994). The final point of consideration is a possible underestimation of root mass. Robinson (2004) has pointed out that the root biomass of large trees is often underestimated by as much as 40%. We have taken this factor into account and adjusted our methods accordingly.

Climate data for each observation was added to the database using the latitude, longitude and elevation for each site. Specifically, we overlaid the site geographic coordinates with climatic data from New et al. (2002) in a Geographic Information System (GIS) to generate detailed climatic information for each individual record. The

climate database contains monthly measures for multiple variables such as: precipitation (mm/month), sunlight (percent of day length with full sunlight) and relative humidity (percent) (New et al. 2002). These measurements were used to determine the amount of water and light availability at a site. In addition, a principal components analysis was used to generate a PC axis that represented a natural gradient in the combination of precipitation, sunlight and relative humidity. It is important to note that these measurements portray the amount of sunlight and water available at a site; the amount available to any given plant will also be affected by factors such as competition with surrounding plants and soil attributes. The climatic data is taken from about 30 years over a 10-minute grid.

Intraspecific analyses

Intraspecific patterns between organ mass tradeoffs and environmental variables were examined for *Cryptomeria japonica* (Thunb. Ex L. f.) D. Don, which was well represented in the dataset in terms of having a suitable sample size and a large range of environmental conditions. Since optimal partitioning theory naturally implies tradeoffs, Principal Components Analysis was used to determine the tradeoffs in form and function. Tradeoffs in organ mass for each individual were calculated by performing a Principal Components analysis on the correlations of log leaf, stem and root mass. The first PC axis was an equal loading for the three organ masses, accounting for the increase in size, and was therefore not used in the analyses. The remaining two PC axes encompassed the tradeoffs between the organ masses. These axes were saved and compared to the log of

total precipitation, percent of full sunlight received at a site (representing water and light availability) and relative humidity. Additionally, a PC axis of these log transformed environmental variables that would decrease transpiration and water stress for plants (high precipitation and relative humidity and low sunlight) was created to address the effect of a natural gradient of multiple environmental conditions on biomass allocation. Analyses were performed with JMP IN 5.1 statistical software (JMP statistical software, SAS institute).

Interspecific analyses

The relationship between interspecific tradeoffs in biomass allocation and environmental variation was also evaluated to determine whether species adapted to different environmental conditions exhibit optimal partitioning patterns. The PCA values were obtained for each species following the methods above and the mean value for each species was used. The mean PCA values were then compared to the mean of log precipitation, sunlight and relative humidity as well as the environmental PC axis encompassing a natural gradient of these three variables. The sites represented in the data span a 3500 cm/yr precipitation, 70% sunlight, and 45% relative humidity gradient. These analyses were performed with JMP IN 5.1 statistical software (JMP statistical software, SAS institute).

Independent contrasts were used to remove any potential statistical effects due to phylogeny for the interspecific analyses. Independent contrasts were performed using phylocom (<http://www.phylodiversity.net/phylocom/>; Webb et al. 2004). The

phylogenetic tree for the seed plants was produced using PHYLOMATIC (Webb and Donoghue 2003), with branch lengths added in phylocom. Phylomatic uses the phylogenetic information from the Angiosperm Phylogeny website (Stevens 2001 onwards) for the backbone of the tree, which bases phylogenies on the most recent molecular analyses of Angiosperms and nodes having greater than 70% bootstrap values.

Results

Variation in intraspecific biomass partitioning allometries

The PC axes encompassed the tradeoffs between organ masses in the loadings (Table 1). For example, in *Cryptomeria japonica*, the second PC axis loaded strongly positive for leaf mass and negative for stem and root mass, indicating a tradeoff between photosynthetic and non-photosynthetic mass. In *C. japonica*'s third PC axis, root mass had a strong, negative loading and stem mass had a strong, positive loading, suggesting a fairly even tradeoff between stem and root mass within the non-photosynthetic mass components. Recall that the first PC axis had equal positive loadings for the three organ masses, indicating an increase in total size, and therefore was not used in the analyses.

The PC axes for *Cryptomeria*

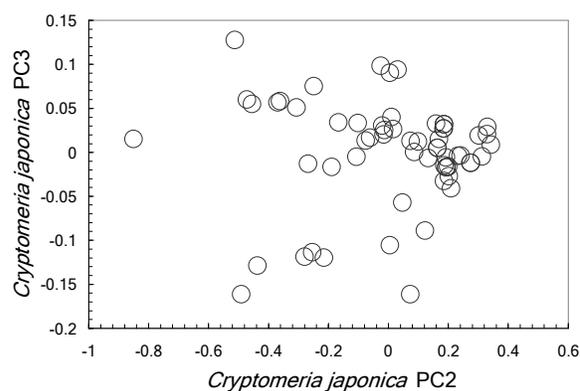


Fig. 1. PC2 and PC3 plotted in bivariate space for *Cryptomeria japonica*. PC2 and PC3 reflect tradeoffs in biomass allocation between leaf, stem and root mass. PC1 had equal loading for the three biomass components and therefore encompassed an increase in total size. PC2 and PC3 are orthogonal to each other and therefore independent.

japonica (Figure 1) represented a tradeoff between photosynthetic and non-photosynthetic mass (PC2) and above and below ground mass (PC3). Both PC2 and PC3 were positively correlated with precipitation and relative humidity (Table 2; Figure 2a-d), demonstrating that leaf mass increases with higher precipitation and humidity at the expense of stem and root mass. Within the non-photosynthetic mass, PC3, root mass decreases and stem mass increases with precipitation and humidity. Similarly, both PC2 and PC3 were negatively related to the amount of sunlight (Figure 2e and f), such that stem and root mass increased with increased sunlight at the expense of leaf mass, and root mass increased at the expense of stem mass. The combination of increased precipitation and humidity with decreased sunlight produced higher leaf mass compared to non-photosynthetic mass, PC2, and a greater amount of stem mass over root mass, PC3 (Figure 2g and h).

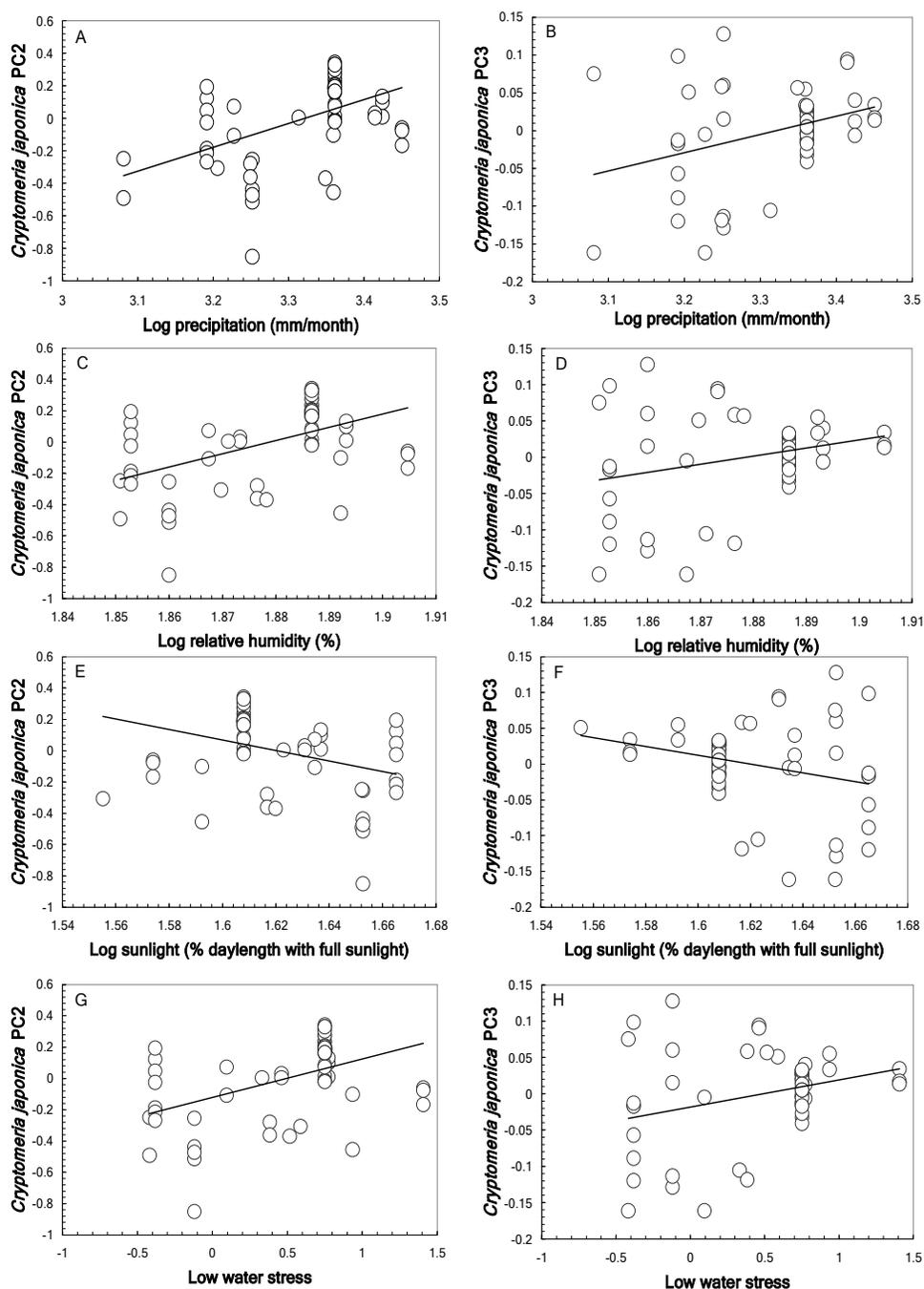


Fig. 2. The relationships for *Cryptomeria japonica* PC2 and PC3 with A-B) log precipitation (mm/month) C-D) log relative humidity (%) E-F) log sun (% day with full sunlight) and G-H) the first PC axis of log precipitation, relative humidity and sunlight expressing a natural gradient of increasing precipitation, relative humidity and decreasing sunlight, which would lead to decreasing water stress. PC2 represents an increase in leaf mass at the cost of stem and root mass. PC3 represents an increase in stem mass at the cost of decreasing root mass.

Variation in interspecific biomass partitioning allometries

Optimal partitioning was also examined interspecifically. The PC2 and PC3 for all seed plants (Table 1; Figure 3) was compared to the mean in log total precipitation, sunlight, relative humidity and a PC axis of these factors that should decrease water stress. There was no relationship between mass PC2 and PC3, with precipitation ($p = 0.79$, $p = 0.65$ respectively), sunlight ($p = 0.96$, $p = 0.46$ respectively), relative humidity ($p = 0.59$, $p = 0.82$ respectively) or the environmental PC axis ($p = 0.81$, $p = 0.64$ respectively).

After accounting for phylogeny, the log of the amount of sunlight, relative humidity and the combination of increasing precipitation and humidity with decreasing sunlight still were not related to variation in PC2 ($p = 0.37$, $p = 0.52$ and $p = 0.99$ respectively) or PC3 ($p = 0.45$, $p = 0.42$ and $p = 0.66$ respectively). However, contrasts for PC3 were negatively related to the contrasts in log precipitation ($R^2 = 0.12$, $p = 0.0109$), indicating that stem mass increases at the expense of root mass interspecifically with increases in precipitation.

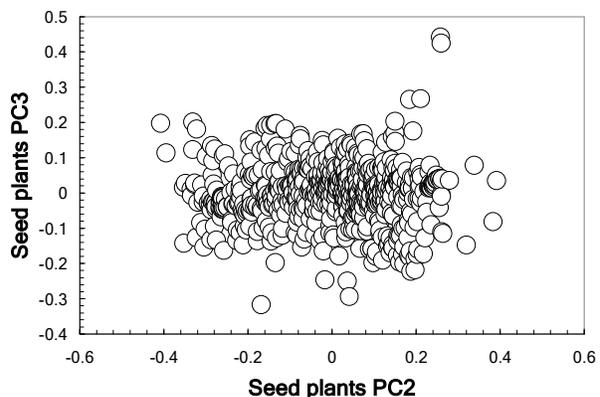


Fig. 3. PC2 and PC3 plotted in bivariate space for all seed plants. PC2 and PC3 reflect tradeoffs in biomass allocation between leaf, stem and root mass. PC1 had equal loading for the three biomass components and therefore encompassed an increase in total size. PC2 and PC3 are orthogonal to each other and therefore independent.

Discussion

Earlier studies in OPT examined the trends between the root/shoot ratio and resource availability. Although this partitioning of organ function is generally correct, other studies have demonstrated that each plant organ performs multiple functions (Körner 1994) and plant organs and their functions are highly integrated (Santiago et al. 2004). Most OPT studies have considered the shoot (leaf plus stem) the part of the plant that acquires sunlight and CO₂ and the root as the part that acquires water and nutrients. However, while stem mass may be largely photosynthetic in low growing herbs, in larger plants stem mass is involved in transport of water and nutrients (plumbing), support of additional leaf mass and positioning leaf mass higher in the canopy to increase light acquisition. Additionally, because of the integrated nature between water transport and photosynthesis (Santiago et al. 2004), environments that cause higher transpiration rates would not only favor decreased leaf mass to reduce transpiration, but should simultaneously favor increased roots to absorb more water. Additionally, increased transpiration or drought conditions might support either a decrease in stem mass, due to decreased height and numbers of branches, or an increase in stem mass, for a given height, to supply numerous small transport routes (i.e. increased density to reduce cavitation). Here we assume that stem mass is primarily related to an increase in height and number of branches to increase light acquisition. Looking within the plumbing (i.e. stem and root mass), root mass may have higher pressures than stem mass given root mass is absorbing and transporting and stem mass is only transporting. The integrated

nature of plant organs is taken into account by using principal component axes of biomass allocation to represent the dominant tradeoffs.

Intraspecific biomass partitioning

Cryptomeria japonica thoroughly followed the expectations of OPT. Leaf mass increased at the expense of stem and root mass (plumbing) with increasing precipitation, indicating increased investment in photosynthesis with higher water availability. Additionally, within the organs involved in transport (i.e. stem and roots), stem mass increased with increased precipitation, therefore allowing increased light acquisition at the expense of water acquisition. The same patterns were observed with increased relative humidity and the combination of increased precipitation and relative humidity with decreased sunlight. The complete opposite pattern was observed for increased sunlight (Table 2), indicating increased investment in water obtainment and plumbing, particularly roots, at the expense of photosynthesis. The relationships between allocation and sunlight were the weakest of the environmentally driven allocation relationships. This may be because the amount of sunlight an individual receives will predominantly be affected by competition and density. Nevertheless, even after accounting for body size, *C. japonica* clearly allocates biomass away from roots and towards light acquisition with increased water availability and lower water stress, and away from leaves and light acquisition with increased light availability and higher water stress.

Interspecific biomass partitioning

Optimal partitioning patterns also occurred interspecifically for precipitation after correcting for evolutionary history. Stem mass increased over root mass, with increases in precipitation, indicating that species that live in habitats with more rain will decrease root mass in favor of light acquisition. Conversely, optimal partitioning patterns did not appear with sunlight, relative humidity and decreased water stress.

It becomes increasingly important to consider other factors that affect resource acquisition when looking for optimal partitioning interspecifically. Plants can change not only their biomass in response to environmental limitations, but also leaf and root surface areas per unit mass, hydraulic conductance, rates of processing, overall morphology or even their life history (Davidson 1969; Jackson, Mooney and Schulze 1997; Reich et al. 1998; Wright et al. 2001; Reich 2002; Santiago et al. 2004). The more factors that are altered to allow plants to acquire limiting resources, the less we would expect large trends to be observed at the global scale.

Changes in leaf morphology are widespread in the literature (Reich 2002). Specific leaf area (SLA) tends to be higher in environments with lower light availability and lower in high light environments. Additionally, SLA is positively related to the maximum photosynthetic rate, therefore an increase in SLA in response to lower light will increase photosynthesis per unit leaf mass (Reich et al. 1997). The patterns between SLA and leaf physiology indicate that a minor change in leaf morphology can have large effects on plant function without a change in biomass allocation.

Recent studies have also indicated the importance of wood density to resource use. Wood density is negatively correlated with the photosynthetic rate per unit leaf area due to constraints on stomatal conductance, and is therefore negatively related to the minimum water potential a plant can support (Santiago et al. 2004). This suggests that plants in hot, dry environments, which would have higher transpiration rates, should have increased stem density to reduce the risk of cavitation.

There are a suite of traits that may also be altered to increase acquisition of limiting resources, such as leaf and root morphology, physiology, growth form, turnover rates and metabolism (Davidson 1969; Jackson et al. 1997; Reich et al. 1998; Reich 2002; Santiago et al. 2004; Wright et al. 2001). Interspecifically, there is much more variation in these traits in response to environmental variation, as well as which traits are altered, compared to intraspecifically. Having so many factors that can influence whole plant function and performance in response to environmental variation may limit our ability to detect optimal partitioning interspecifically. Additionally, plants growing in a natural environment are also exposed to constant fluctuation in resource supply, which may make it difficult to see an influence of optimal partitioning when looking globally (Shipley and Mezaine 2002). Given the factors that limit our ability to find OPT, the fact that we find optimal partitioning at all indicates that it is indeed a significant component of biomass allocation. While Reich (2002) only found that 3 of 26 species demonstrated higher leaf mass fractions in response to low light, there was a consistent shift to stem mass away from root mass, which would also be consistent with optimal partitioning. Other studies have also demonstrated that biomass partitioning does respond to

environmental variation after size is accounted for, however other factors, such as leaf and root surface area, are equally important (Jackson et al. 1997; Reich et al. 1998; Bloor and Grubb 2004).

Conclusions

Optimal partitioning patterns appear both intraspecifically and to a lesser extent interspecifically. These results highlight a couple of interesting points. First of all, variation caused by environmental factors can be integrated with the variation due to large changes in total mass to obtain a more complete prediction of how plants should allocate biomass in response to both changes in size and environment. Secondly, while optimal partitioning is observed, exact patterns may vary from species to species obscuring large-scale interspecific patterns. This variation in biomass allocation may be a result of altering multiple and or different physiological and morphological factors in response to environmental variability, suggesting the need to include a suite of traits to examine in response to environmental change (Reich 2002). This does not suggest that species do not allocate biomass optimally, merely that what is optimal for one species may not be optimal for another. While one species may primarily allocate increased biomass to roots in response to decreasing precipitation, another may increase root surface area, increase the density of stem mass and decrease SLA and leaf mass. Both species are altering form to incur a change in function. As our understanding of the integrated nature of plant form and function continues to grow, so should our definition of what constitutes optimal allocation.

Acknowledgements

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Tables

Table 1. Eigenvector loadings for leaf, stem and root mass from principal component analyses for *Cryptomeria japonica* and all seed plants.

Species	PC2 loadings	PC3 loadings
<i>Cryptomeria japonica</i>	Leaf: 0.82	Root: -0.72
	Stem: -0.42	Stem: 0.70
	Root: -0.39	Leaf: 0.02
All seed plants	Leaf: 0.81	Stem: -0.75
	Stem: -0.48	Root: 0.66
	Root: -0.33	Leaf: 0.09

The PCA was performed on the correlations of log leaf, stem and root mass. The first principal component contained equal loadings for stem, leaf and root mass, indicating a change in size, and were therefore not used in these analyses. The second and third PC axes are columns 2 and 3 respectively. Note, the loading are in order of magnitude.

Table 2. The predicted direction and results of correlations between PC 2 and 3 for *Cryptomeria japonica* with the log of precipitation, relative humidity and sunlight and decreased water stress.

	Log Precipitation	Log Relative Humidity	Log Percent Sunlight	Decreased Water Stress
Predicted Direction				
PC2	<i>Positive</i>	<i>Positive</i>	<i>Negative</i>	<i>Positive</i>
<i>Cryptomeria</i>	<i>Positive</i>	<i>Positive</i>	<i>Negative</i>	<i>Positive</i>
<i>japonica</i>	$R^2 = 0.24$	$R^2 = 0.24$	$R^2 = 0.11$	$R^2 = 0.20$
PC2	$p < 0.0001$	$p < 0.0001$	$p = 0.0088$	$p = 0.0003$
Predicted Direction				
PC3	<i>Positive</i>	<i>Positive</i>	<i>Negative</i>	<i>Positive</i>
<i>Cryptomeria</i>	<i>Positive</i>	<i>Positive</i>	<i>Negative</i>	<i>Positive</i>
<i>japonica</i>	$R^2 = 0.12$	$R^2 = 0.08$	$R^2 = 0.07$	$R^2 = 0.09$
PC3	$p = 0.006$	$p = 0.0300$	$p = 0.0406$	$p = 0.0206$

Decreased water stress represents environmental PC axis encompassing a natural gradient of increasing precipitation and relative humidity with decreasing sunlight. The predictions for PC2 and PC3 are based on optimal partitioning theory. The results for the PC axes with each environmental variable contains the direction of the correlation (italicized), the R^2 and the p value.

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APPENDIX C

ASSESSING THE RELATIVE EFFECTS OF PHYLOGENY, ENVIRONMENT AND
GROWTH FORM ON PLANT BIOMASS ALLOCATION AND LEAF PHENOLOGY

Megan C. McCarthy and Brian J. Enquist

Summary:

- Studies on plant biomass allocation have mainly focused on the effects of environmental differences, while the effects of evolutionary history have been relatively ignored. Furthermore, the role of plant size has been shown to be considerable. Here, we assess the relative contribution of environment, growth form and phylogeny on variation in biomass allocation, after accounting for size, and leaf traits.
- We determine the amount of variation in biomass allocation, after accounting for changes in size, due to phylogeny, growth form and nutrient level for 48 species of eudicots grown in two different nutrient levels. Additionally, we examine which traits are phylogenetically conserved.
- We demonstrate that when biomass allocation is examined across numerous species, phylogeny was the strongest factor after the effects of size are considered. Although phylogeny explains a large fraction of the variability, neither biomass allocation nor leaf traits appear to be significantly conserved in the evolution of these clades, suggesting this is the result of more recent selection.
- The combination of these results suggests that a large-scale understanding of plant biomass allocation across diverse groups must consider the effects of both phylogeny and plant size.

Key words: plant allometry, biomass allocation, residual variation, phylogenetic conservatism, specific leaf area, growth form

Introduction:

Plants display tremendous diversity in both form and function. The factors that control how plants partition biomass between stems, roots and leaves have been explored for more than a century (Kny 1894; Pearsall 1927), and remains an important focus in many areas of ecology and evolutionary theory (Grime 1979; Tilman 1988; Bernacchi *et al.* 2000; Iwasa 2000). The preponderance of studies have been intraspecific with changes in one or more environmental factor, with a few interspecific studies examining growth form (Monk 1966; Mooney 1972; Tilman 1988). So, not surprisingly, most studies have emphasized that much of the variation in biomass allocation results from environmental differences (Davidson 1969; Hunt & Burnett 1973; Bloom *et al.* 1985; Hunt & Nichols 1986). However, recent studies have highlighted that size largely effects allocation patterns and may explain much of the variation attributed to environment (Coleman *et al.* 1994; McConnaughay & Coleman 1999; Bernacchi *et al.* 2000). In addition, the effects of evolutionary history have been relatively ignored, despite its importance to many other areas of plant form and function.

The drastic influence of size has been seen in many areas of plant and animal biology (Peters 1983; Calder 1996; West *et al.* 1999). Recently, its importance has extended to biomass allocation in plants (Coleman *et al.* 1994; McConnaughay & Coleman 1999; Bernacchi *et al.* 2000; Enquist & Niklas 2002). Rooted in metabolic scaling theory, Enquist & Niklas (2002) demonstrated that seed plants follow very similar rules for biomass distributions. Based on the constraints of transporting resources from the ground

through the plant and the photosynthetic harvesting capacity of the leaves, they predicted that:

$$M_L = \beta_1 M_S^{3/4} \quad (1)$$

$$M_L = \beta_2 M_R^{3/4} \quad (2)$$

$$M_S = \beta_3 M_R^1 \quad (3)$$

where M_S , M_R , and M_L are stem, root, and leaf mass respectively and the Beta terms are normalization constants. The beta values reflect differences in biomass distribution not accounted for by size (further detail on the model in Enquist & Niklas 2002; Niklas & Enquist 2002).

Extensive data for arboreal conifers, monocots and eudicots spanning six orders of magnitude generally fit the predicted relationships. Plant size explained 97 to 99% of the variation in biomass distribution to leaf, stem, and root mass (Enquist & Niklas 2002). However, there existed one to two orders of magnitude residual variation around these allometric scaling relationships. So, although plant size accounts for much of the variation in organ mass distribution there still remains a significant amount of residual variation to be explained by other factors.

Environmental variation is certainly one factor that may account for the variation left after size is considered. Indeed, several studies have shown the influence of environmental variation, even after size is accounted for (McConnaughay & Coleman 1999; McCarthy & Enquist in press). However, very little emphasis has been placed on the role of evolutionary history on patterns and plasticity of biomass partitioning.

Phylogeny appears to be significant to many areas of plant form and function (Antunez *et*

al. 2001; Bloor & Grubb 2004) including plasticity of biomass partitioning (Osone & Tateno 2005). However, we still do not know how much variability in biomass allocation is even available for a plastic response to environmental variability. Additionally, it is unclear whether the variation that is constrained by evolutionary history is highly conserved, meaning it is largely explained by early divergences in the phylogeny, or is the result of more recent selection pressures.

Other factors such as plant growth form and leaf traits may also influence allocation patterns. Previous studies have found differential biomass partitioning dependent on growth form (Monk 1966; Körner 1994; Tilman 1988). While some of these studies indicate that large woody plants have the highest root to shoot ratios (Monk 1966) and others suggest that annuals have the highest root to shoot ratios (Tilman 1988), few of these studies account for body size which varies greatly between growth forms. Additionally, leaf traits, such as specific leaf area and photosynthetic rate, have been shown to be related to allocation patterns (McCarthy *et al.* 2007 in press). Leaf morphology and physiology often adjust in response to environmental conditions (Reich 2002; Wright *et al.* 2001; Bloor & Grubb 2004). Increasing specific leaf area (SLA), the maximum photosynthetic rate (A_{max}) or the amount of leaf area for a given size (LAR) allows a plant to increase carbon acquisition without changes in biomass allocation. Therefore these traits may interact with allocational patterns.

The goal of this study is to combine the perspectives used in the different biomass partitioning studies. We examine how evolutionary history, growth form and nutrient level affect variation in biomass allocation not determined by size, by looking at the

residual variation from allometric regressions. This allows us to quantify how much of the variation not accounted for by size is due to evolutionary history, growth form and nutrient differences. In addition, we can assess whether the variation due to phylogeny is conserved, meaning most of the variation is early in the phylogeny, or more recent. By determining the amount of variation due to size, phylogeny and growth form, we can essentially determine how much variation is available for plastic responses to both biotic and abiotic factors not accounted for by this study. Specifically, we analyzed: (i) the relative importance of evolutionary history, growth form and nutrient level on the residual variation after controlling for size; (ii) the relative importance of evolutionary history, growth form and nutrient level of leaf morphology and physiology; and (iii) the phylogenetic conservativeness in biomass allocation, leaf morphology and leaf physiology.

Materials and Methods:

Experimental set-up:

Eight genera from four large Angiosperm eudicots clades, Rosid I, Rosid II, Asterid I, and Asterid II were chosen based on having two species from each growth form, annual, herbaceous perennial and woody perennial, for a total of 48 species (Table 1). All of the individuals were started from seeds from USDA, and started within a month of each other based on predetermined germination rates. Plants were grown in a hydroponic set-up in which water was continuously circulated throughout the system. Each species was grown in two different nutrient levels in a randomized block design, full strength and quarter

strength Hoagland's solution, with micronutrients being the same in both treatments. Three individuals from each species and each treatment were harvested at four different times during the course of a year, for a total of 1152 individuals, to generate a large size gradient.

Before harvesting, the photosynthetic rate was measured with a Licor 6400 for several leaves throughout the canopy level at full irradiance to calculate the maximum photosynthetic rate (A_{\max}), and leaf area per unit leaf mass (SLA) was measured. Following these measurements, plants were divided into leaf, stem and root components and dried for 48 hours. Leaf area ratio (LAR) was calculated by regressing the log of total leaf area and against total size, and the residual variation of the relationship was used to remove size dependence.

Statistical analyses:

The effects of size were removed from the biomass allocation patterns by using the residual variation from the allometric scaling relationships. The biomass allocation regressions for leaf, stem and root mass, from which the residuals were obtained, were performed against total mass minus the response variable to create independence (Fig. 1). Additionally, the measurement for total leaf area for a given size (LAR) was determined by regressing leaf area against total size and using the residual variation from this relationship. The residual variation from the biomass partitioning and leaf area relationships are independent of size so all further analyses are looking at the variation after the effects of size are removed. The specific leaf area (SLA) and the photosynthetic

potential (A_{\max}) were independent of plant size, therefore these measurements were simply log transformed.

Relative contribution of nutrient level, growth form and phylogeny:

The percent of variation in specific leaf area, photosynthesis per unit leaf mass, leaf area ratio and residual variation in biomass allocation by nutrient level, growth form and phylogeny was determined using a multiple regression analysis. The variation due to phylogeny was determined from a patristic distance matrix, a matrix of distances between all of the tips in the phylogeny. The phylogenetic tree used for this analysis was generated in phylomatic (<http://www.phylodiversity.net/phylomatic/>) and branch lengths were added in Phylocom. Phylomatic uses the Angiosperm Phylogeny (Stevens 2001 onwards), which is based on recent molecular phylogenetic analyses and nodes with >80% Bootstrap support. The node ages are determined from the fossil record (<http://www.phylodiversity.net/phylocom/>; Webb *et al.* 2006). The phylogenetic distance matrix is put into a Principle Components Analysis to determine the axes of variation (Desclaves *et al.* 2003). A mixed stepwise regression is used to determine which axes are important to the trait of interest (SLA, A_{\max} , LAR, leaf, stem and root residuals). Only the significant principle component axes ($p < 0.05$) are used in the multiple regression analysis. The amount of variation from nutrient level, growth form and phylogeny is calculated using the sum of squares from each component divided by the total sum of squares, with any non-significant factors removed from the model. This

allows quantification of each of these factors on the variation in leaf traits and biomass allocation not accounted for by size.

Phylogenetic Trait Conservatism:

Traits in which much of the variability is explained by early divergences indicate that the trait is phylogenetically conserved (Niklas 1994; Nealen & Ricklefs 2001; Guo *et al.* 2003). The program phylocom (Webb *et al.* 2006) was used to determine the degree of trait conservatism for SLA, Amax, LAR and leaf, stem and root mass residual variation. The means of the traits or trait residuals for each species were used in the analyses. Phylocom provides a measure of trait conservatism which is essentially determined by examining the magnitude of contrasts across the tree (<http://www.phylodiversity.net/phylocom/>; Webb *et al.* 2006). If most of the divergences between related species have similar trait values, then the phylogenetic signal is stronger and indicates a more conserved trait. A nonconserved trait should have large trait differences near the tips of the tree and smaller trait differences in the vicinity of the root. Significance testing for trait conservatism is based on 1000 randomizations of the trait values across the tips. Phylocom further provides the amount of variation explained by each bifurcation in the tree. This data was examined, along with the age of each node, to provide additional information on the patterns of trait variation.

Results:

Relative contribution of nutrient level, growth form and phylogeny:

Total size explained 83 to 91 percent of the total variation in allocation to leaf, stem and root mass in addition to the total leaf area (Fig. 1; $R^2 = 0.8334$, $p < 0.0001$; $R^2 = 0.8468$, $p < 0.0001$; $R^2 = 0.9123$, $p < 0.0001$ and $R^2 = 0.8380$, $p < 0.0001$ respectively). Multiple regression analyses were then used to determine how much of the variation in SLA, A_{\max} , LAR and the residual variation in biomass allocation was the result of phylogeny, growth form, and nutrient level. When considered jointly with phylogeny, growth form was not significant for any of the traits examined except LAR (Table 2), suggesting that differences due to growth form may primarily be phylogenetic in origin. Phylogeny explained more than half of the variation in leaf, stem and root mass that was not explained by size as well as 10 to 30 percent of the variation in SLA, A_{\max} and LAR. Nutrient level explained a significant amount of variation in leaf and root mass residuals as well as SLA, A_{\max} and LAR. However, this only accounted for less than one percent of the variation in root mass residuals and SLA and less than five percent for leaf mass residuals, A_{\max} and LAR (Table 2).

Phylogenetic Trait Conservatism:

None of the traits were significantly conserved phylogenetically. In fact the opposite was seen, such that there was a negative relationship between the amount of variation at each node and the age of the node (Fig. 2) for stem and root mass residuals as well as for the A_{\max} and LAR ($R^2 = 0.2992$, $p = 0.0348$; $R^2 = 0.3282$, $p = 0.0256$; $R^2 = 0.3848$,

$p=0.0136$ and $R^2 = 0.2580$, $p=0.0532$ respectively), suggesting the variability is due to recent selection pressures.

Discussion:

The importance of body size to form and function has become evident for both animals (Peters 1983; Calder 1996; West *et al.* 1999) and plants (Niklas 1994). Not surprisingly, plant biomass allocation is also primarily governed by the total mass (Enquist & Niklas 2002; Niklas & Enquist 2002). Given that the majority of biomass partitioning studies have shown environmental factors to be important (Davidson 1969; Hunt & Burnett 1973; Hunt & Nichols 1986; Iwasa 2000), this might suggest that much of the remaining variation should be subject to environmental conditions. However, factors such as environmental history have received very little attention despite its importance to other areas of plant form and function (Antunez *et al.* 2001; Bloor & Grubb 2004). In this study, we examine the relative effects of nutrient level, growth form and phylogeny on the variability in biomass allocation not accounted for by size for 48 species of eudicots grown hydroponically.

Relative contribution of nutrient level, growth form and phylogeny:

More than half of the variation was explained by phylogeny in leaf, stem and root mass, after removing the effects of size (Table 2). Phylogeny only explained 12 - 28% of the variation in SLA, A_{\max} and LAR, but was still the largest contributor to these factors. This supports a previous study of a global dataset containing a diverse collection of

conifers, monocots and dicots, which demonstrated that phylogeny explained the majority of the variation in biomass allocation (McCarthy *et al.* in press). Of further interest was that growth form was only a significant factor for LAR in the multiple regression analyses, suggesting that the influence of growth form on biomass allocation is actually resulting from differences in size and evolutionary history. The nutrient level had no effect on stem mass allocation and a negligible effect on root mass and SLA. The largest effect of nutrient level was on leaf mass, leaf area ratio and photosynthetic rate – traits that are important to carbon acquisition and therefore growth rate.

Although much of the variability was encompassed by phylogeny, there was still a significant amount of variation due to the nutrient treatment. Interestingly, each of the factors that had the most variation explained by the nutrient level are beneficial to carbon acquisition, highlighting the importance of this function. These results are similar to another study which found that plasticity of crown form traits was greater than plasticity in biomass allocation (Bloor & Grubb 2004). Given that the highest plasticity appears to be in traits associated with carbon gain, this suggests that a) being able to change the carbon acquisition ability is extremely important to fitness and b) it is more optimal to have several moderately plastic traits than one extremely plastic trait. The additional result that phylogeny explained over half of the variation in biomass allocation, but less of the variation in leaf traits further suggests that these traits may be more plastic in response to environmental variation.

Of further interest was that 35-60% of the variation in leaf, stem and root mass as well as total leaf area that was not explained by size, remained unexplained after

considering phylogeny, growth form and nutrient level. Yet, if you consider the vast amount of variation explained by size itself, 83-91%, this essentially only leaves between 4-10% of the variation of these traits unexplained. This could easily be due to measurement error or slight variations in the light availability. However, 79% of the total variation in photosynthetic potential and 87% of the total variation in SLA was not explained by phylogeny, growth form or nutrient differences, which seems excessive to contribute to measurement error or slight differences in conditions. This suggests that there are other factors unaccounted for. Since we only examined the influence of nutrients, it may be that environmental differences in light and water, both of which have been shown to be important for these traits (Reich 2002; Wright *et al.* 2001), may account for much of this residual variation. Other factors could include herbivory, competition or a number of other abiotic and biotic factors. Regardless, this indicates that SLA and Amax may demonstrate much more plasticity in response to abiotic and biotic factors than biomass allocation.

Phylogenetic Trait Conservatism

Given the large amount of variation in biomass allocation explained by evolutionary history, it is of interest to determine whether this variation is the result of traits being constrained early in the evolution of the taxa or from recent selection pressures. The latter appears to be the case. Neither allocation to stems, roots and leaves nor leaf morphology and physiology are conserved. In fact, quite the opposite occurs for stem and root mass as well as the photosynthetic potential per unit leaf mass and leaf area

ratio, suggesting that much of the variation is due to more recent selection pressures. So although biomass allocation does not appear to express tremendous plasticity in response to proximate factors, it appears to be evolutionarily plastic.

It is important to point out, that the terms biomass allocation and biomass partitioning have also been used to refer to different species or populations adapted to particular environmental conditions, such as shade or drought (Chapin 1980, Givnish 1988, Tilman 1988). Given that much of the variation in stem and root biomass and LAR and A_{\max} is more recent, much of the phylogenetic variation may be due to recent selection pressures based on the resource gradient these species are largely exposed to. So, instead of a plastic response to environmental variability, populations in natural environments may primarily show an adaptive response to the regular environmental conditions with some plasticity to respond to more microenvironmental changes.

Conclusions:

The important contribution of body size has been demonstrated in the form and function of animals (West *et al.* 1999) as well as plants (Niklas 1994; Enquist & Niklas 2002; Niklas & Enquist 2002). The majority of biomass partitioning studies have concentrated on environmental effects (Davidson 1969; Hunt & Burnett 1973; Hunt & Nichols 1986; Iwasa 2000), while largely ignoring the importance of constraints due to evolutionary history.

Our results indicate that phylogeny explains the majority of the variation in biomass partitioning after accounting for size. Although phylogeny largely explains the patterns

in allocation and leaf phenology, these traits are not phylogenetically conserved. In fact, the variation appears to be from more recent selection pressures. Our results have important implications for ecological studies. Understanding interspecific differences in organ mass allocation must first assess constraints due to plant size and evolutionary history. Future studies that incorporate phylogeny and size with environmental conditions will be able to provide further insight into the ecological and evolutionary processes that shape differences in botanical biomass allocation.

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Tables

Table 1 Eight genera of Eudicots each containing two species of annual, perennial and woody perennial growth forms.

Genus	Annual	Perennial	Woody Perennial
Erysimum	<i>E. cheiranthoides</i>	<i>E. asperum</i>	<i>E. linifolium</i>
Erysimum	<i>E. repandum</i>	<i>E. inconspicuum</i>	<i>E. capitatum</i>
Helianthus	<i>H. annuus</i>	<i>H. grosseserratus</i>	<i>H. giganteus</i>
Helianthus	<i>H. agrophyllus</i>	<i>H. mollis</i>	<i>H. niveus</i>
Hibiscus	<i>H. cannabinus</i>	<i>H. laevis</i>	<i>H. acetosella</i>
Hibiscus	<i>H. trionum</i>	<i>H. lasiocarpus</i>	<i>H. striatus</i>
Linum	<i>L. grandiflorum</i>	<i>L. altaicum</i>	<i>L. campanulatum</i>
Linum	<i>L. usitatissimum</i>	<i>L. flavum</i>	<i>L. lewisii</i>
Nicotiana	<i>N. attenuata</i>	<i>N. alata</i>	<i>N. excelsior</i>
Nicotiana	<i>N. rustica</i>	<i>N. sylvestris</i>	<i>N. glauca</i>
Potentilla	<i>P. biennis</i>	<i>P. argentea</i>	<i>P. arguta</i>
Potentilla	<i>P. norvegica</i>	<i>P. erecta</i>	<i>P. fructosa</i>
Salvia	<i>S. sclarea</i>	<i>S. sylvestris</i>	<i>S. officinalis</i>
Salvia	<i>S. viridis</i>	<i>S. x superba</i>	<i>S. splendens</i>
Solanum	<i>S. rostratum</i>	<i>S. cardiophyllum</i>	<i>S. melongena</i>
Solanum	<i>S. sisymbriifolium</i>	<i>S. pinnatisectum</i>	<i>S. pseudocapiscum</i>

Table 2 Amount of variation in leaf mass, stem mass, root mass, specific leaf area, photosynthetic potential and LAR explained by growth form, nutrient level and phylogeny.

Characteristic	Growth form	Nutrient level	Phylogeny	Residual
Leaf Mass	NS	4.3%	52.8%	42.9%
Stem Mass	NS	NS	65.2%	34.8%
Root Mass	NS	0.5%	50.3%	49.2%
SLA	NS	0.6%	12.2%	87.24%
PSN/Mass	NS	4.0%	16.8%	79.2%
LAR	2.7%	4.3%	28.9%	64.1%

Note. The amount of variation in leaf, stem and root mass as well as LAR is the variation remaining after size is accounted for. The residual variation is the variation not explained by the model.

Figures

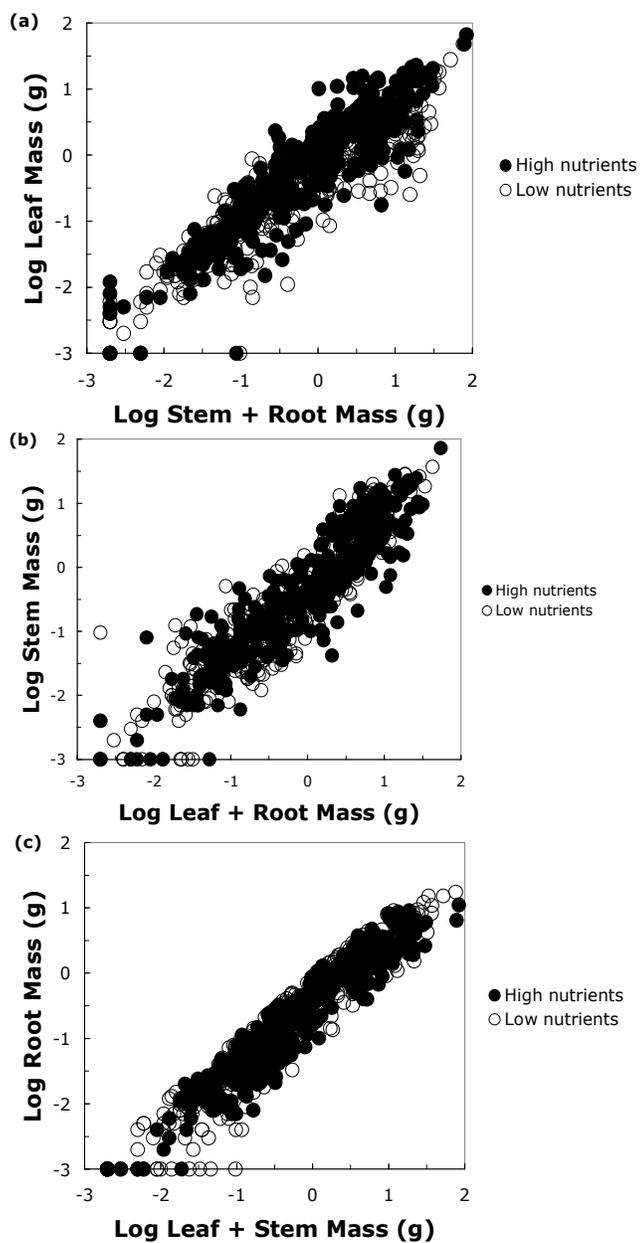


Fig. 1. Biomass allometric relationships between a) leaf, b) stem and c) root mass. Each component is regressed against total mass minus that component (i.e. leaf mass by stem + root mass) to create independence. Size explains 83% of the variation in leaf, 85% of the variation in stem and 91% of the variation in root mass.

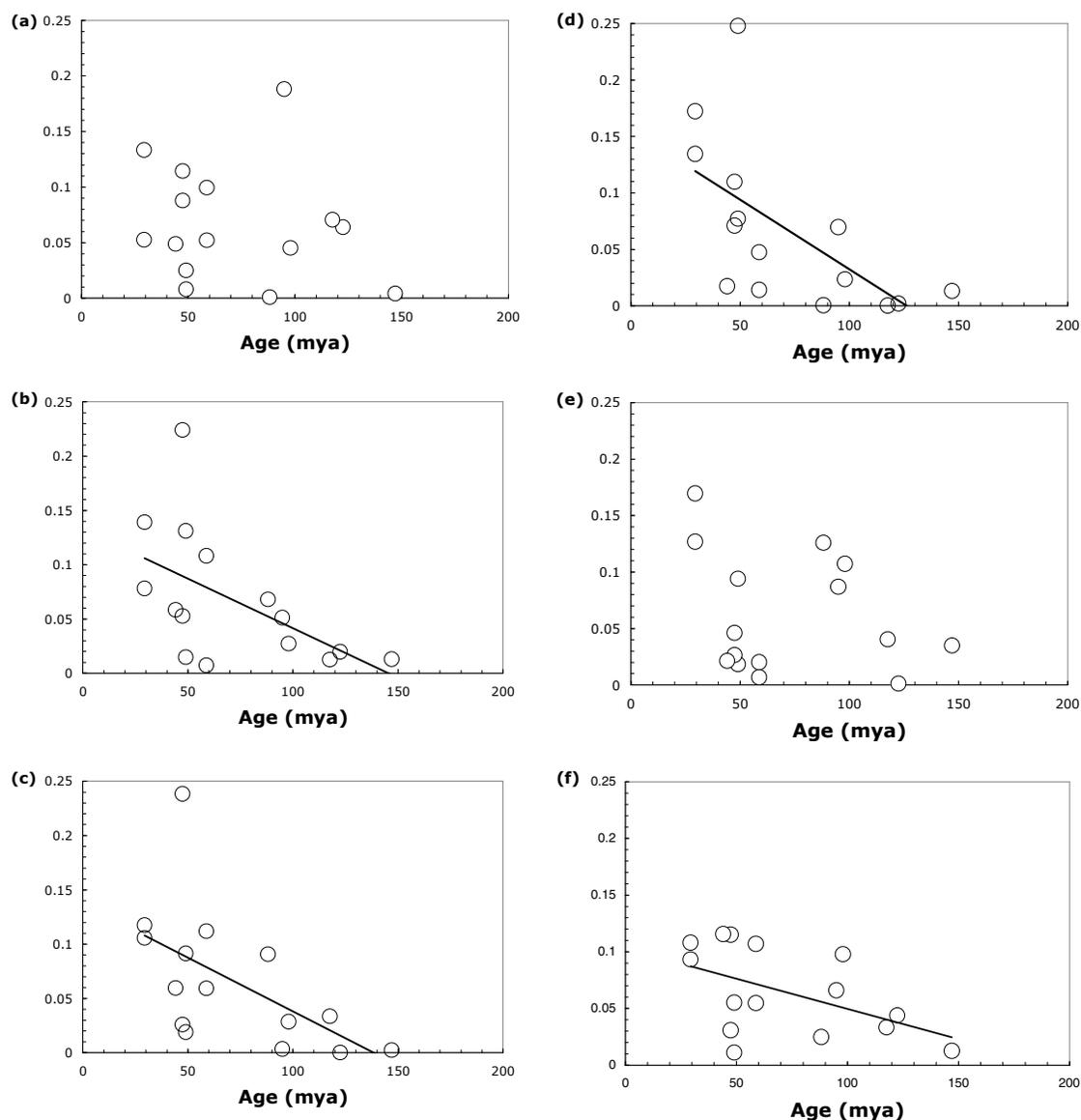


Fig. 2. The relationship between the age of a node and the amount of variation explained by that bifurcation for a) leaf, b) stem and c) root mass as well as d) A_{\max} , e) SLA and f) LAR. A positive relationship would suggest that the traits are fairly conserved. A negative relationship indicates that most of the variability due to phylogeny is the result of more recent selection pressure.

APPENDIX D

TESTING ALLOMETRIC AND OPTIMAL PARTITIONING MODELS IN
PATTERNS OF BIOMASS ALLOCATION: THE ROLE OF NUTRIENT LEVEL
AND SPECIES-SPECIFIC DIFFERENCES

Megan C. McCarthy and Brian J. Enquist

Abstract

Optimal Partitioning Theory (OPT) indicates that patterns of biomass allocation should vary according to environmental conditions. Essentially, plants should allocate biomass to the organ that acquires the most limiting resource. However, the generality of OPT has recently been challenged as to how much of this variation is due to changes in size. Further, the role of evolutionary history is relatively unknown. An alternative approach, Allometric biomass Partitioning Theory (APT), has recently been proposed to predict how plants partition metabolic production based on the constraints of body size. APT predicts that leaf, stem and root mass should be scale to body size according to physiological and morphological constraints. Here we conduct a broad-scale experiment in order to examine patterns of organ partitioning while accounting for allometrically driven biomass allocation. Plants of differing evolutionary histories and growth forms are grown hydroponically in two different nutrient levels, to not only account for optimal partitioning and size, but also differences in evolutionary history. The majority of species demonstrated some response to changes in nutrient level. After controlling for the influence of plant size, 80% of species responded to changes in nutrient level according to the predictions of OPT, where allocation to leaf and stem mass occurred at the expense of root mass. Interestingly, the species-specific allocation response to nutrient level was not uniform, indicating the influence of evolutionary history. Because of the effect of phylogeny, OPT patterns were much stronger intraspecifically. Additionally, 65% of the species demonstrated an allometric response. This study indicates that patterns of biomass allocation, in response to environmental change is likely general, but yet

multifaceted. By viewing plant responses as potentially a function of size and/or allocation reveals several potential environmental response strategies.

Key words: biomass allocation, environmental variation, optimal partitioning, plant allometry, evolutionary history.

Introduction

Plants have a limited amount of energy to divide between the multiple functions an individual must perform. Therefore, allocation to any one component must come at the expense of the other functions. The diversity of plant growth forms suggests there are many ways that plants can partition the energy that is put into biomass. How biomass is partitioned between leaves, stems and roots are important to many areas of ecology and evolutionary biology (Grime 1979; Tilman 1988; Bernacchi et al. 2000; Iwasa 2000).

Multiple studies have suggested that variability in biomass partitioning is primarily due to environmental differences. These studies primarily fall under Optimal Partitioning Theory (OPT), which suggests that plants should allocate biomass to the organ that acquires the most limiting resource (Thornley 1972; Bloom, Chapin and Mooney 1985). Plants exposed to higher light should partition more biomass to roots at the expense of above-ground mass and plants exposed to higher moisture partition more biomass to above-ground mass at the expense of root mass. There is a large body of literature that generally supports OPT (Crist and Stout 1929; Davidson 1969; Hunt and Burnett 1973). OPT has been generally accepted as an explanation for how plants respond to variable environments, and is a cornerstone of many theories in plant ecology and evolution (Grime 1979; Tilman 1988; Westoby 1998).

However, the generality of OPT has come under scrutiny. Various studies have illustrated that the partitioning effects attributed to environmental differences in many earlier studies, were, to some extent, the result of not accounting for differences in size (Coleman, McConnaughay and Ackerly 1994; Coleman and McConnaughay 1995;

McConnaughay and Coleman 1999; Reich 2002). By using the ratio of root mass to shoot mass as a measure of the relative amount of investment between these differing organs, an increase in size, due to the addition of a resource, may appear to change partitioning when root and shoot mass do not scale isometrically (i.e. with an exponent $>$ or $<$ 1). Therefore, changes in size may explain some of the variability in biomass allocation that was previously attributed to environment (Coleman, McConnaughay and Ackerly 1994; Coleman and McConnaughay 1995; Bernacchi et al. 2000; Müller, Schmid and Weiner 2000).

While the majority of OPT studies focus on differences in above relative to below-ground mass, without necessarily separating leaf and stem mass function (Körner 1994), variation in other traits may change in order to increase acquisition of a limiting resource without changes in biomass. An increase in the amount of leaf area for a given leaf mass (SLA), potential photosynthetic rate (A_{\max}), or the amount of leaf area for a given plant mass (LAR), can increase carbon and light acquisition relative to water and nutrient acquisition (Wright et al. 2004). Additionally, these factors have been shown to change in response to changes in particular environmental conditions (Wright et al. 2001; Reich 2002; Bloor and Grubb 2004). Therefore, physiological and other morphological changes may be just as important as allocational differences to compensate for environmental discrepancies. Due to the role of size and the potential role of other morphological and physiological traits, it is unclear whether OPT is a general explanation for the observed variation in biomass partitioning, and if OPT can adequately describe the response to environmental differences when all of these factors are combined.

An alternative biomass partitioning model, Allometric biomass Partitioning Theory (APT), has recently been developed to predict how plants should optimally divide their metabolic production between leaves, stems, and roots with changes in size (Enquist and Niklas 2002; Niklas and Enquist 2002). This model is based on allometric theory, which examines how organismal attributes change with body size according to the allometric equation: $\log Y_1 = \log \beta_0 + \alpha \log Y_2$, where β is the allometric constant, α is the scaling exponent, and Y_1 and Y_2 are interdependent variables for size (Peters 1983). This equation can also be written in the form of a power-law such that $Y_1 = \beta Y_2^\alpha$. APT is based on the constraints of maximizing photosynthetic harvesting capacity and resource transport, while minimizing hydrodynamic resistance and transport times. The allometric partitioning model predicts that leaf mass, M_L should scale with stem mass, M_S , and root mass, M_R . Specifically, $M_R \propto M_S \propto M_L^\theta$ where for plants larger than seedlings Enquist and Niklas provide arguments that $\theta \sim 3/4$. Thus, leaf mass should scale allometrically with stem and root mass but that stem mass should scale isometrically to root mass such that:

$$M_L = \left(\frac{1}{\beta_{13}} \right) M_R^\theta \quad (1)$$

$$M_L = \left(\frac{1}{\beta_{11}} \right) M_S^\theta \quad (2)$$

$$M_S = \left(\frac{\beta_{12}}{\beta_{13}} \right) M_R^1 \quad (3)$$

where the Beta terms are allometric constants that may vary across species or differing environments (Enquist and Niklas 2002; Niklas and Enquist 2002).

Conifers, monocots and dicots spanning 12 orders of magnitude fit the predicted relationships remarkably well (Enquist and Niklas 2002; Niklas and Enquist 2002).

However at any given whole-plant mass, there were one to two orders of magnitude variation, which may reflect species-specific and habitat differences (Enquist and Niklas 2002; Niklas and Enquist 2002).

While it appears reasonable that size explains much of the variability in biomass allocation, given that many areas of both plant and animal form and function are size dependent (Peters 1983; Calder 1996), it also seems logical that environmental differences should affect allocation. If low light levels are limiting survival and reproduction, plants that are able to increase allocation to leaf mass, or stem mass for plants limited by competition, would have higher fitness. Yet increasing leaf surface area or photosynthetic rate would also allow plants to increase carbon acquisition. Indeed, changes in leaf morphology and physiology do appear to adjust in response to environmental conditions (Reich 2002; Wright et al. 2001), and may even compensate for decreased morphological plasticity (Davidson 1969; Bloor and Grubb 2004). In addition to size and leaf traits, phylogeny also seems to be extremely important to biomass allocational patterns. For example, McCarthy et al. (2007) demonstrated that evolutionary history explained over a third of the variation in organ partitioning, that was not accounted for by size, in a global dataset. Additionally, phylogeny explained half of

the variability in biomass allocation after size was accounted for in this study (McCarthy and Enquist submitted).

The allometric partitioning model (Enquist and Niklas 2002; Niklas and Enquist 2002) provides a theoretical baseline from which to understand the effects of environmental variation. While allometric partitioning and optimal partitioning have been treated as alternate ideas (Shipley and Meziane 2002), they may also be used as complimentary to explain biomass partitioning patterns in plants. By examining the variability in biomass partitioning after accounting for size, one can assess how environmental conditions affect allocation. In this study, we examined how two different nutrient levels affected variability in biomass allocation, after accounting for size. We focused on 48 species that were picked due to phylogenetic and functional group similarities and differences. We assessed OPT by hypothesizing that, independent of evolutionary history or function type, plants should allocate biomass in response to differences in a limiting resource. If plants are allocating biomass optimally, then individuals grown in the high nutrient treatment should have less biomass allocated to root mass, and more to stem and leaf mass, then plants of the same size grown in the low nutrient treatment. To determine if leaf morphology and physiology were also being adjusted in response to environmental differences, perhaps instead of biomass, we measured specific leaf area (SLA), potential photosynthetic rate (A_{\max}) and leaf area ratio (LAR). Specifically, we address: 1) whether optimal partitioning patterns occur intraspecifically in biomass allocation after accounting for size, 2) whether leaf

morphology and physiology change intraspecifically in response to different nutrient levels and 3) whether these patterns holds interspecifically.

Methods

Experimental set-up

Eight genera from four large Angiosperm eudicots clades (Rosid I, Rosid II, Asterid I, and Asterid II) were chosen based on having two species from each of three growth forms (annual, herbaceous perennial and woody perennial) (Table 1). All of the individuals were grown from seed, which were ordered from USDA. All species were started within a month of each other based on predetermined germination rates. Plants were grown in a hydroponic set-up in which water was continuously circulated throughout the system and nutrient levels were checked regularly. Individuals were arranged in a randomized block design and grown in two different nutrient levels, full strength and quarter strength Hoagland's solution, with micronutrients being the same in both treatments. A total of 12 individuals from each species and each treatment were harvested at different times during the course of 9 months, to generate a large size gradient, for a total of 1152 individuals.

Before harvesting, the photosynthetic rate was measured at full irradiance, using a Licor 6400, for several leaves throughout the canopy level, to calculate the maximum photosynthetic rate (A_{max}). Specific leaf area (SLA) was also measured for several leaves at different heights of the canopy. Following these measurements, plants were divided into leaf, stem and root components and dried for 48 hours so leaf, stem and root mass

could be determined. The amount of leaf area for the total mass of the plant, leaf area ratio (LAR), was also calculated from the biomass and leaf morphology measurements by regressing total leaf area by total mass and using the residual variation from that relationship to remove any size dependence.

Intraspecific analyses

Patterns of biomass allocation were examined intraspecifically for species having at least one order of magnitude size variation and seven individuals per treatment. An extra sum-of-squares test was used to determine how much the treatment contributed, when treatment had a significant effect, to the variation in allocation not explained by size. First, a regression was performed for each species on leaf, stem, root and above ground mass against the total mass, minus the dependent variable to create independence, with all masses log transformed (i.e. log leaf mass against log stem plus root mass). Next a multiple regression was performed for leaf, stem, root and above ground mass, with the nutrient treatment added into the explanatory variables. The sum of squares that were not explained by the nutrient treatment model were subtracted from the error sum of squares from the main regression, and this was divided by the total sum-of-squares to determine how much more variation was explained by the nutrient treatment. This percentage was then divided by the total variation not explained by the simple size model. This determined how much of the variation not explained by size, was explained simply due to differences in the nutrient level. A least-squares (LS) regression model was used to determine the variation around the relationship related to size since we were specifically

interested in variation in the dependent variable, error in the independent variable was small and the correlation coefficients were high.

An additional multiple regression was performed for leaf, stem, root and above ground mass for each species with an interaction term between total mass and nutrient treatment added into the explanatory variables to determine if the nutrient treatment had any affect on the slope of the biomass scaling relationships. For species where there was a significant interaction between nutrient treatment and mass, this model was used as the full model in the extra sum-of-squares test in place of the multiple regression with just nutrient level. The difference in slopes conveyed by this model was verified by testing for differences between the reduced major axis (RMA) regression slopes for each species grown in each nutrient level. The RMA regression exponent value is equal to the LS exponent divided by the correlation coefficient, r . Therefore, RMA and LS are very similar when r is high. RMA is recommended in determining the functional relationship when variables are interdependent and error is expected to be similar (Sokal and Rohlf 1995).

An allometric effect of nutrient treatment was determined by using a t-test to determine whether individuals within a given species grown in high nutrients were larger than individuals grown in the low nutrient treatment. Additionally, a one-way ANOVA was used to determine the effect of nutrient level on leaf morphology and physiology. The amount of variation due to nutrient level was examined for each species on SLA, A_{\max} and the LAR. Following optimality, we expected SLA, A_{\max} and LAR to increase

in response to higher nutrient levels in order to increase carbon acquisition with greater amounts of resources.

Interspecific analyses

The effects of nutrient level on patterns of biomass allocation and leaf traits were also examined interspecifically to determine whether the patterns remained the same. The mean for leaf, stem, root, above ground and total mass as well as SLA, A_{\max} , and LAR were determined for each species in each treatment level. The effects of nutrient level were essentially determined the same way they were in the intraspecific methods, except the variables for each species were averaged for each treatment. Regression analysis was used to determine how much size explained of the variation in leaf, stem, root and above ground mass. A multiple regression examined the additional effect of nutrient level using an extra sum-of-squares test. The allometric response to nutrient level was again tested using a t-test and the effects of nutrient level on SLA, A_{\max} and LAR were determined using a one-way ANOVA. All analyses were performed with JMP IN 5.1 statistical software (JMP statistical software, SAS institute).

Results

Variation in intraspecific biomass partitioning allometries

The amount of variation, unaccounted for by size, in allocation to above ground, leaf, stem and root mass due to the nutrient treatment was examined for 20 species (Table 2). Fifty-five percent of the species demonstrated increased allocation to above-ground mass

in the higher nutrient treatment ($p < 0.05$) (Figure 1), with another 15% exhibiting the same trend ($p < 0.10$), as would be expected based on optimal partitioning theory. This increase in above ground mass allocation accounted for almost 80% of the variation, after accounting for size, in *Hibiscus lasiocarpus*. Each species divided biomass between above and below-ground mass in a different way. Only 35% of the species showed increased allocation to leaf mass at the higher nutrient levels, with another 10% showing a trend and one species showing the opposite effect. However, 45% of the species showed increased allocation to stem mass in the high nutrient treatment, as optimal partitioning would predict. Root mass was less in the high nutrient treatment for 45% of the species, with 10% more showing a trend toward decreasing root mass (Tables 2 and 4).

In addition to the partitioning response, 65% of the species demonstrated an allometric response (Table 3), with 50% being significant ($p < 0.05$) and the other 15% showing a trend ($p < 0.10$). While there was quite a bit of nutrient related variation in size and biomass allocation, there was much less variation in leaf traits due to the nutrient treatment (Tables 3 and 4). The greatest response to the nutrient level was with LAR, with 25% of the species having an increased LAR at the high nutrient treatment ($p < 0.05$), and another 15% showing that same trend ($p < 0.10$). However, this increase did explain between 15 to 50% of the variation in LAR for those species. Only 15% of the species exhibited increased photosynthetic rates due to higher nutrients, usually accounting for around half of the variability in A_{\max} , with an additional 15% exhibiting a

trend. Only 10% of the species showed a trend of increased SLA in the high nutrient treatment, which only explaining about 14% of the variation in SLA.

Variation in interspecific biomass partitioning allometries

Variation in biomass allocation, after accounting for size, was also examined interspecifically for all 48 species. These patterns were similar to what was found intraspecifically. In the high nutrient level above-ground mass, mostly driven by leaf mass, was higher, while there was a trend for lower root mass. The nutrient treatment explained 6.2% of the variation, that was not explained by size, in above-ground mass ($p = 0.0156$), 5.0% of the variation in leaf mass ($p = 0.0307$) and 2.8% of the variation in root mass ($p = 0.1089$). Neither SLA nor A_{\max} were affected by the nutrient treatments ($p = 0.6150$, $p = 0.2238$), however LAR was higher in the high nutrient treatment ($R^2 = .069$, $p = 0.0124$). Despite the lower response in partitioning and leaf traits, there was still a moderate allometric response across species ($R^2 = .170$, $p = 0.0067$).

Discussion

The large discrepancy between allometric studies, which suggest that biomass allocation is largely a factor of size, and experimental studies, which suggest that variability in biomass allocation is largely a factor of environmental variability, indicates that both size and environmental differences are important to biomass allocation and should be examined jointly. The seemingly great differences between these two types of studies are at least partly explained by the approaches. Allometric studies tend to look

over generous size ranges. Indeed this is a necessity for allometric studies, since slopes will be more sensitive at small size ranges. However as a result, the variation around the allometric relationship will tend to be small compared to the size span, which determines much of the variation. However, if the size range examined is fairly small, i.e. less than one order of magnitude, the variation due to other factors will appear much greater (McCarthy et al. 2007). In addition, partitioning studies that only examine root to shoot ratio may not account for allometric changes when root and shoot mass do not scale isometrically.

In addition to body size and environmental effects, there are likely other factors that will determine allocation patterns. Differences in evolutionary history appear to produce much of the disparity in biomass allocation patterns (McCarthy et al. 2007; McCarthy and Enquist submitted). Additionally, alterations in other morphological and physiological traits may lead to similar functional modifications as changes in allocation. Therefore, adjustments in other traits may compensate or supplement the changes that would be necessary in biomass allocation to lead to a similar difference in function. We attempt to integrate these factors by examining how much more variation in biomass allocation is explained by differences in nutrient level, after accounting for changes in size, as well as examining changes in SLA, A_{\max} and LAR.

Intraspecific biomass partitioning

Across 20 species of Eudicots of various growth forms, only four species did not show any optimal partitioning in response to nutrient levels, after accounting for differences in

size (Table 4). For the most part, differences in nutrient level explain 25-50% of the variation in allocation that was not explained by size. However, nutrient level contributed up to 79% of the variation for some biomass components in *Hibiscus lasiocarpus*. While 80% of the species demonstrated some form of optimal partitioning, the way in which each species partitioned biomass varied. Only four species increased allocation to leaf and stem mass while decreasing allocation to root mass in response to higher nutrients. The other species either demonstrated a tradeoff between stem or leaf mass with root mass, only increased stem and leaf mass, or only modified allocation to one organ (Table 2). These differences in allocation may be related to the natural habitat of each of the species (Osone and Tateno 2005). A taxa that is predominantly in a low light environment would have a greater fitness benefit by putting energy into increased leaf mass to increase carbon acquisition, while a taxa that lives in a higher light environment with more competition should gain a greater fitness benefit by adding stem mass to keep its canopy exposed to sunlight.

Changes in biomass allocation were predominantly seen in variability around the scaling relationship, the residual variation, and not the slope. While a few species did show significant changes in their slope, this only comprised less than 10% of the species for any given scaling relationship. Additionally, this was likely due to small size ranges and/ or sample size. Many studies examining intraspecific allometric changes in allocation due to environmental effects naturally do not tend to span large size ranges, and this study is no exception to this pattern. Although each species in its entirety spanned at least one order of magnitude of total size, the size span within a nutrient

treatment varied due to an allometric effect of nutrient treatment (Tables 3 and 4). For the majority of the species, the individuals from the low nutrient treatment only spanned the smaller end of the size spectrum covered by the individuals in the high nutrient treatment (Figure 1). Therefore, the size ranges tended to vary between the treatments. Additionally, it is unclear how large a size span is necessary before sensitivity in the slope is removed. However, this is likely larger than one order of magnitude and will also depend on the sample size.

In contrast to the large response of both biomass partitioning and allometry to nutrient treatment, there was very little response in the leaf traits (Tables 3 and 4). The changes that did occur appeared to have a phylogenetic pattern. For example, all of the *Potentilla* species showed an increase in LAR with increased nutrients, while the *Hibiscus* species had increased A_{\max} . However, SLA did not significantly increase with nutrient levels for any of the species.

The lack of variation in leaf traits in response to nutrient treatment is intriguing. On the one hand, many studies have demonstrated that leaf morphology and physiology are integral parts of plant form and function, and therefore often respond to environmental variability (Reich 2002; Wright et al. 2001). In fact, some studies have shown leaf and canopy morphology can show a greater response to environmental differences than biomass partitioning (Reich 2002; Bloor and Grubb 2004). On the other hand, these traits may respond to variation in light or water availability much more than they respond to variation in nutrients. For example, the plasticity of leaf traits can depend on the treatment (Reich 2002; Bloor and Grubb 2004) and the strategy of a given

species (Reich et al. 2003). For example, understory canopy species tend to be more conservative and therefore less plastic, while opportunistic species may demonstrate much higher plasticity to environmental conditions (Müller et al. 2000). Additionally, the amount of plasticity in a trait can also depend on the natural conditions of the species' native habitat (Osone and Tateno 2005). One might expect root characteristics to change more in response to nutrient level than leaf traits. However, due to the hydroponic set-up, root morphology did not tend to vary. All of the root mass was essentially fine root mass, and was therefore best examined simply by the differences in total root mass for a given plant size. It would be informative to do further experiments examining changes in other abiotic factors.

Interspecific biomass partitioning

Evolutionary history appears to be a large component of biomass allocation for seed plants (McCarthy et al. 2007) and explains over half of the variation in partitioning patterns, not explained by size, for this particular experiment (McCarthy and Enquist submitted). Therefore, it is interesting to determine whether the intraspecific patterns hold when looking across species. While many of the optimal partitioning patterns held, they were much weaker, i.e. explained a smaller portion of the variation and had a lower p value, in the interspecific analyses. The primary explanation for this difference is that although the majority of the species demonstrated optimal partitioning, the method of doing so varied between species (Table 2). The response of a species to a change in nutrients may vary depending on the strategy or niche the species is adapted to (Muller et

al. 2000). In addition, since phylogeny explains over half of the variation in allocation to leaf, stem and root biomass after accounting for size, only a small part of the variation remains to be explained interspecifically. Yet despite the small amount of variation available to be explained, given the dominating effects of size and phylogeny, nutrient level still significantly affects allocation to above-ground biomass allocation, indicating it is still an important, though small, factor (Figure 2).

A moderate allometric response occurred in response to increased nutrient levels. Individuals grown in the higher nutrient levels were larger than those grown in the low nutrient levels, following what was found intraspecifically. However, neither SLA nor A_{\max} were related to nutrient availability interspecifically. This is not surprising given that these patterns were not prevalent intraspecifically (Table 3). However, the amount of leaf area for a given body size does change with changes in nutrient level even when examined interspecifically. Although nutrient treatment only explained about 7% of the variation in LAR, almost 30% of the variability in LAR is already explained by evolutionary history (McCarthy and Enquist submitted). Most likely the response of the leaf traits will depend on the environmental condition that is changing.

Conclusions

Many of the species demonstrated both a plastic response to an increase in nutrients in addition to an allometric response. Optimal partitioning patterns were present both intraspecifically and to a lesser extent interspecifically, similar to an earlier study examining a worldwide forestry dataset (McCarthy and Enquist 2007). These

results suggest that differences in biomass partitioning related to environmental factors can be integrated with allometric partitioning changes to gain a more comprehensive understanding of how plants should allocate biomass in response to both changes in size and environment. Secondly, while optimal partitioning is observed, precise relationships may vary between species, lessening large-scale interspecific patterns. This variation is primarily caused by different strategies, such as opportunistic or conservative, among species, indicating that optimality is species-specific and perhaps specific to the environmental variable.

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Figures

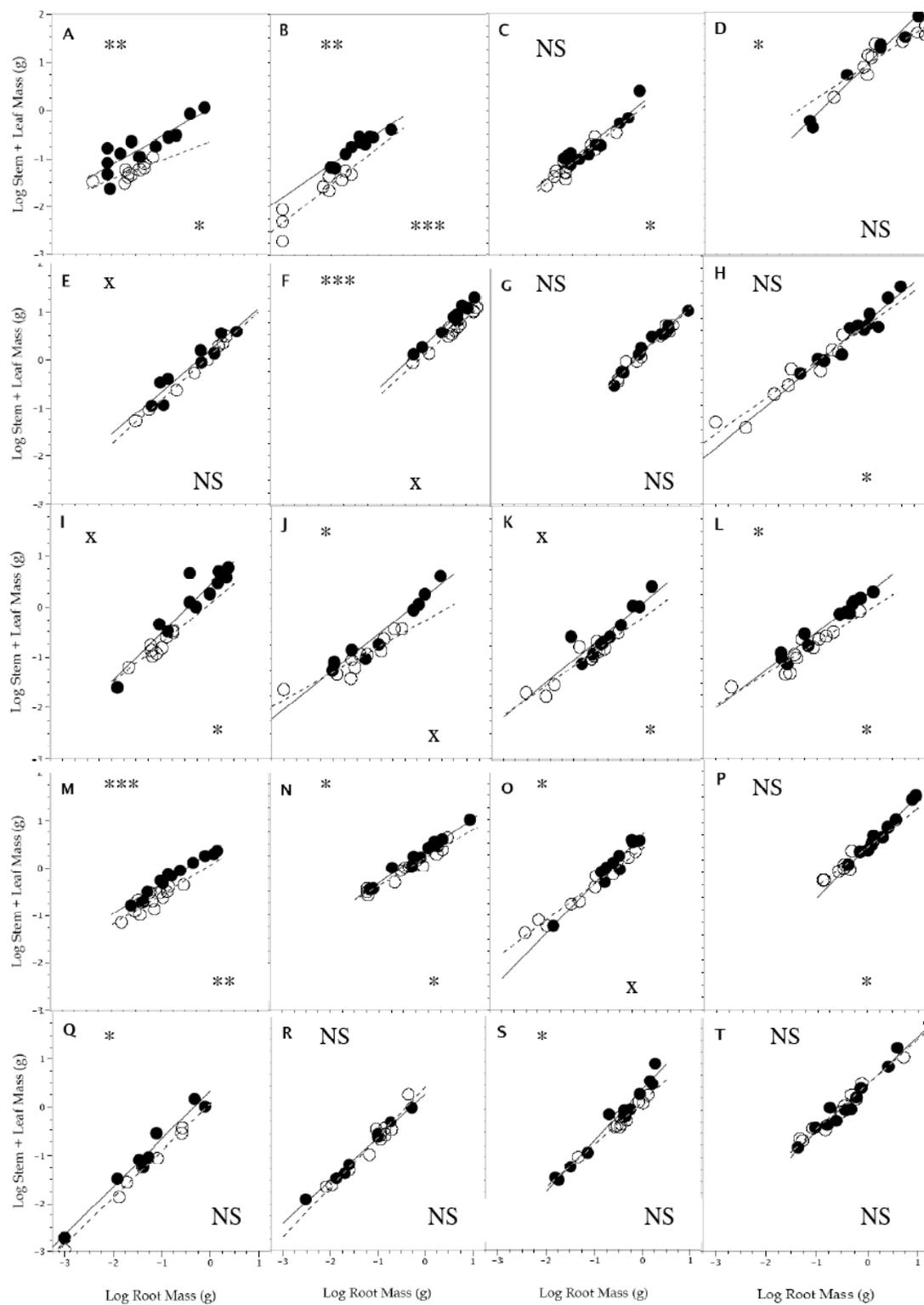


Fig 1. Allometric scaling relationships between the log of above ground biomass to the log of below ground biomass for 20 species of Eudicots grown in two different nutrient levels. X and Y axes are scaled the same for each of the species. Filled in circles with a solid line represent the high nutrient treatment (full strength Hoagland's solution) and hollow circles with a dotted line represent the low nutrient treatment (1/4 strength Hoagland's solution). The letters in the upper left hand corner of each graph correspond to the letter listed in the label column of Table 2. The symbols (NS, x, *, **, ***) in the upper left hand corner of each graph corresponds to the significance level (not significant, trend of $p < 0.10$, $p < 0.05$, $p < 0.001$ and $p < 0.0001$ respectively) of the partitioning response to increased nutrients. The symbols in the lower right hand corner of each graph corresponds to the significance level of the allometric response.

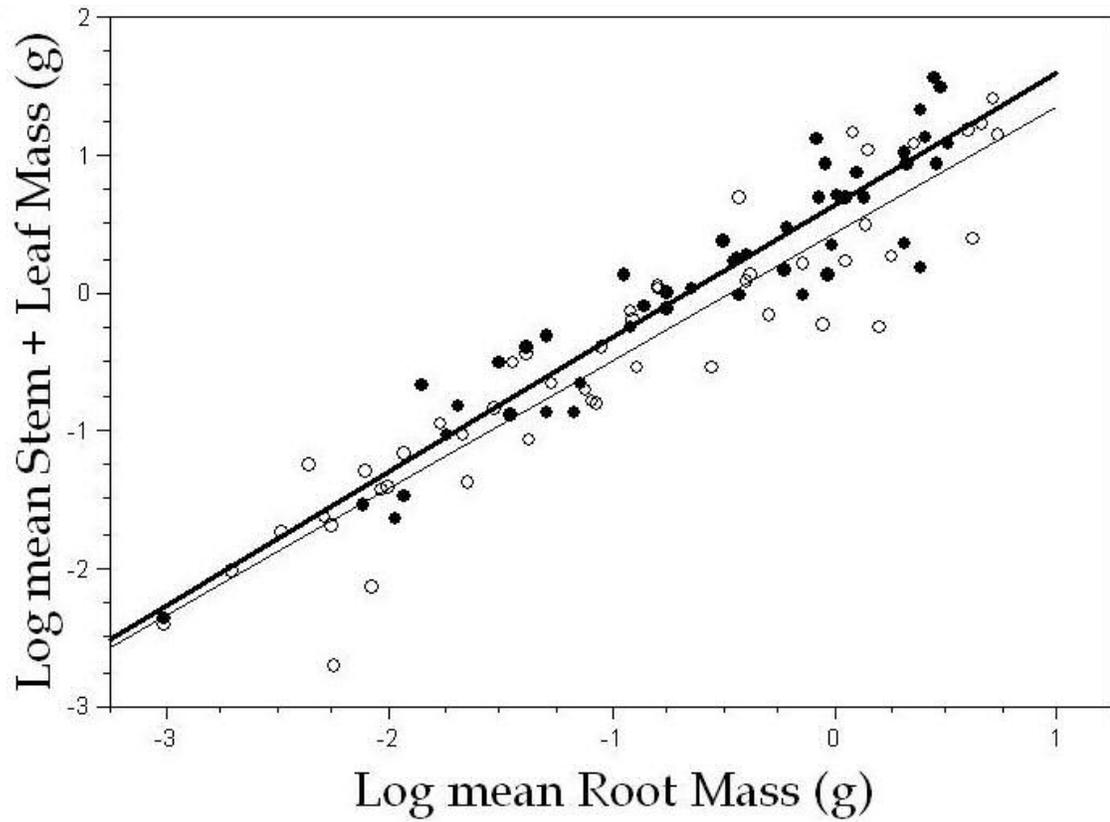


Fig 2. Interspecific allometric scaling relationship between the log of above ground biomass to the log of below ground biomass for 20 species of Eudicots grown in two different nutrient levels. Each point is the mean value for each taxa in each nutrient treatment. Filled in circles with a solid line represent the high nutrient treatment (full strength Hoagland's solution) and hollow circles with a dotted line represent the low nutrient treatment (1/4 strength Hoagland's solution).

Tables

Table I: Species from each Genus Separated into Annual, Perennial and Woody

Perennial Growth Forms

Genus	Annual	Perennial	Woody Perennial
Erysimum	<i>E. cheiranthoides</i>	<i>E. asperum</i>	<i>E. linifolium</i>
Erysimum	<i>E. repandum</i>	<i>E. inconspicuum</i>	<i>E. capitatum</i>
Helianthus	<i>H. annuus</i>	<i>H. grosseserratus</i>	<i>H. giganteus</i>
Helianthus	<i>H. agrophyllus</i>	<i>H. mollis</i>	<i>H. niveus</i>
Hibiscus	<i>H. cannabinus</i>	<i>H. laevis</i>	<i>H. acetosella</i>
Hibiscus	<i>H. trionum</i>	<i>H. lasiocarpus</i>	<i>H. striatus</i>
Linum	<i>L. grandiflorum</i>	<i>L. altaicum</i>	<i>L. campanulatum</i>
Linum	<i>L. usitatissimum</i>	<i>L. flavum</i>	<i>L. lewisii</i>
Nicotiana	<i>N. attenuata</i>	<i>N. alata</i>	<i>N. excelsior</i>
Nicotiana	<i>N. rustica</i>	<i>N. sylvestris</i>	<i>N. glauca</i>
Potentilla	<i>P. biennis</i>	<i>P. argentea</i>	<i>P. arguta</i>
Potentilla	<i>P. norvegica</i>	<i>P. erecta</i>	<i>P. fructosa</i>
Salvia	<i>S. sclarea</i>	<i>S. sylvestris</i>	<i>S. officinalis</i>
Salvia	<i>S. viridis</i>	<i>S. tequicola</i>	<i>S. splendens</i>
Solanum	<i>S. rostratum</i>	<i>S. cardiophyllum</i>	<i>S. melongena</i>
Solanum	<i>S. sisymbriifolium</i>	<i>S. pinnatisectum</i>	<i>S. pseudocapiscum</i>

Note: Each genus contains two species in each of the three growth forms.

Table II: Percent Variation in Biomass Allocation explained by Increased Nutrient Level

Species	Label	AG (↑)	Leaf (↑)	Stem (↑)	Root (↓)
<i>Erysimum capitatum</i>	A	51.3%**	NS	41.5%*	29.0%*
<i>Erysimum inconspicuum</i>	B	50.5%**	NS	24.6%*	22.8%*
<i>Erysimum linifolium</i>	C	NS	28.5%*	NS	NS
<i>Helianthus agrophyllus</i>	D	36.2%*	47.1%*	41.0%*	NS
<i>Helianthus giganteus</i>	E	18.8%	NS	NS	19.9%
<i>Hibiscus lasiocarpus</i>	F	78.9%***	76.4%***	55.0%**	78.0%***
<i>Hibiscus striatus</i>	G	NS	NS	NS	NS
<i>Nicotiana glauca</i>	H	NS	17.8%	NS	NS
<i>Nicotiana sylvestris</i>	I	15.0%	NS	NS	NS
<i>Potentilla argentea</i>	J	25.8%*	NS	27.0%*	16.0%
<i>Potentilla arguta</i>	K	16.8%	17.5%	NS	NS
<i>Potentilla biennis</i>	L	32.5%*	26.4%*	21.6%*	21.3%*
<i>Potentilla erecta</i>	M	55.1%***	NS	28.7%*	37.7%*
<i>Potentilla norvegica</i>	N	34.1%*	18.4%*	NS	28.8%*
<i>Salvia officinalis</i>	O	39.1%*	<u>22.0%*</u>	38.6%*	42.1%*
<i>Salvia sclarea</i>	P	NS	NS	NS	NS
<i>Salvia tequicola</i>	Q	47.4%*	32.1%*	33.9%*	42.2%*
<i>Salvia viridis</i>	R	NS	NS	NS	NS
<i>Solanum pseudocapsicum</i>	S	38.7%*	41.4%**	NS	40.6%**
<i>Solanum sisymbriifolium</i>	T	NS	NS	NS	NS

Note: The arrows next to each of the top row variables indicate the direction predicted by OPT. The label column identifies the individual species graphs in Figure 1. A single asterisk next to the percent of variation explained by increased nutrients indicates $p < 0.05$, a double asterisk $p < 0.001$, triple asterisk $p < 0.0001$ and no asterisk is a trend of $p < 0.10$. Italicized numbers indicate a change in slope and the single underlined number indicates the opposite direction from the optimal partitioning prediction.

Table III: Percent of Variation in Total Size and Leaf Traits explained by Nutrient Level

Species	Size (↑)	SLA (↑)	A _{max} (↑)	LAR (↑)
<i>Erysimum capitatum</i>	29.9%*	14.2%	NS	18.9%
<i>Erysimum inconspicuum</i>	64.0%***	NS	NS	NS
<i>Erysimum linifolium</i>	28.1%*	NS	22.8%	NS
<i>Helianthus agrophyllus</i>	NS	NS	18.2%	NS
<i>Helianthus giganteus</i>	NS	NS	NS	NS
<i>Hibiscus lasiocarpus</i>	17.3%	NS	41.9%*	47.7%*
<i>Hibiscus striatus</i>	NS	NS	49.5%*	NS
<i>Nicotiana glauca</i>	38.8%*	NS	22.3%	NS
<i>Nicotiana sylvestris</i>	44.4%*	NS	NS	NS
<i>Potentilla argentea</i>	15.5%	NS	NS	23.0%
<i>Potentilla arguta</i>	38.8%*	NS	NS	25.9%*
<i>Potentilla biennis</i>	30.3%*	NS	NS	31.6%*
<i>Potentilla erecta</i>	43.5%**	14.5%	NS	34.3%*
<i>Potentilla norvegica</i>	19.6%*	NS	NS	21.5%
<i>Salvia officinalis</i>	17.7%	NS	NS	NS
<i>Salvia sclarea</i>	42.9%*	NS	NS	NS
<i>Salvia tequicola</i>	NS	NS	57.4%*	NS
<i>Salvia viridis</i>	NS	NS	NS	NS
<i>Solanum pseudocapiscum</i>	NS	NS	18.2%	15.5%
<i>Solanum sisymbriifolium</i>	NS	NS	NS	NS

Note: The arrows next to each of the top row variables indicate the predicted direction. A single asterisk next to the percent of variation explained by increased nutrients indicates $p < 0.05$, a double asterisk $p < 0.001$, triple asterisk $p < 0.0001$ and no asterisk is a trend of $p < 0.10$.

Table IV: Percent of Species that Demonstrated the Predicted Response for each Trait.

Trait	Percent Species
Size (↑)	65%
AG (↑)	70%
Leaf (↑)	45%
Stem (↑)	45%
Root (↓)	55%
Allocation	80%
SLA (↑)	10%
A_{\max} (↑)	35%
LAR (↑)	40%

Note: The trait allocation refers to a change in any of the biomass components.