Impact of nutrient heterogeneity on plant response and competition in Coastal plain species

by

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ABSTRACT

Relationships between nutrient heterogeneity, root foraging behavior and short-term competitive interactions were investigated for six species native to southeastern USA. Monoculture, two- and six-species garden plots were established and fertilized to create spatially homogeneous or heterogeneous nutrient conditions. After 3.5 months, root proliferation in rich patches (precision) and aboveground biomass response to heterogeneity were assessed in monocultures, and competitive outcomes (aboveground biomass) were determined from mixed-species plots. In monoculture plots, two species were relatively precise foragers, but no species showed significant aboveground biomass response to nutrient treatment. Correlations between precision and aboveground biomass were weak ($-0.40 < r < 0.17$). In two-species plots, interspecific competition was influenced by soil heterogeneity in two of six cases tested ($P < 0.05$), and precision was the behavior most correlated with competitive success. In six-species plots, spatial pattern of nutrients had no influence on aboveground growth or competition. Results suggest that heterogeneity influences competition, but the influence is context-specific and generally small. Precision may be the foraging behavior that most influences interspecific interactions.

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OVERVIEW AND OBJECTIVES

This work explores the impacts of fine-scale heterogeneity of soil nutrients on two types of plant response: (1) root system morphology and functions related to nutrient uptake (foraging behavior); and (2) plant growth as affected by neighboring plants (intra- and inter-specific competition). Patchiness of the soil environment at the scale of individual plants has been demonstrated in many community types. Since belowground competition may predict plant success, particularly in nutrient limited environments, ecologists have targeted root foraging behavior as an important component determining community composition. Several root foraging mechanisms have been identified and are being studied including: morphological, physiological and demographic plasticity. Debate continues regarding the significance of these mechanisms with respect to a variety of issues including species richness, competition and community structure. However, much of the literature to test these ideas is based upon short-term, highly controlled greenhouse studies involving plant monocultures. Inherently, there is strong value to a long term, multi-species experiment in a field setting.

The first objective of this study was to determine if soil nutrient heterogeneity influences competitive outcomes in early successional forests. My first hypothesis was that because plant species differ in nutrient foraging behavior, soil resource heterogeneity will influence competitive outcomes in early successional forests. To test this hypothesis, monocultures, and mixtures of 2-,3-, 4-, and 6-species were established in Garden Plots in 1998 and 1999 and fertilized to create spatially homogeneous, and heterogeneous soil nutrient conditions. Aboveground biomass was harvested in 1998, 1999 and 2000 to determine effects of nutrient heterogeneity and species composition on competitive outcomes (Chapters 1 and 3). Because of limits imposed by a drought and the lack of automated irrigation (Chapter 1), I was unable to test long-term effects of nutrient heterogeneity on competitive outcomes, and thus I used irrigation and a longer-term experiment (Chapter 3) for a better test of hypothesis 1. I also used Goldberg's index of competition to analyze 4 and 6-species plots to determine community level response to nutrient heterogeneity. My second objective was to determine which foraging mechanisms are most likely related to competitive success when soil nutrients are

heterogeneous, and my second hypothesis was that plants that proliferate roots in nutrient rich patches would have stronger competitive ability in heterogeneous than in homogeneous soils. To test this hypothesis, I measured the degree of root proliferation for each species in the monoculture plots in each garden plot experiment, and compared this to competitive outcomes (Chapters 1 and 3). My third objective was to determine if the plants in my study system use both root proliferation (morphological plasticity) and altered nutrient uptake rates (physiological plasticity) in response to nutrient heterogeneity. I hypothesized that both behaviors occurred in each species. I grew individual plants in pots with either heterogeneous or homogeneous nutrient arrangement, and then used short-term dosing of the plants with low concentrations of ^{15}N labeled ammonium nitrate to determine rates of nitrogen uptake per unit of root mass dosed (Chapter 2). Finally, I used results of these studies to explore relationships between nutrient foraging mechanisms, competitive outcomes, other plant traits including growth form and successional status, and abiotic factors such as water availability.

Chapter One: Does Root Foraging Behavior Influence Competitive Interactions in Soil with Spatially Heterogeneous Nutrients?

ABSTRACT

Relationships between nutrient heterogeneity, root foraging behavior and short-term competitive interactions were investigated for six species native to southeastern USA. Monoculture, two- and six-species garden plots were established and fertilized to create spatially homogeneous or heterogeneous nutrient conditions. After 3.5 months, root proliferation in rich patches (precision) and aboveground biomass response to heterogeneity were assessed in monocultures, and competitive outcomes (aboveground biomass) were determined from mixed-species plots. In monoculture plots, two species were relatively precise foragers, but no species showed significant aboveground biomass response to nutrient treatment. Correlations between precision and aboveground biomass were weak ($-0.40 < r < 0.17$). In two-species plots, interspecific competition was influenced by soil heterogeneity in two of six cases tested ($P < 0.05$), and precision was the behavior most correlated with competitive success. In six-species plots, spatial pattern of nutrients had no influence on aboveground growth or competition. Results suggest that heterogeneity influences competition, but the influence is context-specific and generally small. Precision may be the foraging behavior that most influences interspecific interactions.

Key Words: coastal plain species, interspecific competition, intraspecific competition, precision, root foraging behavior, sensitivity, spatial nutrient heterogeneity

INTRODUCTION

Although universally acknowledged, the idea that soil resources are heterogeneous has not been fully integrated into conceptual models of plant competition. Recent models (e.g., Grime, 1977; Tilman, 1985; Wu & Sharpe, 1985; Keddy, 1989; Grace & Tilman, 1990) incorporate a number of mechanisms that control competitive outcomes, and they vary considerably from descriptive to resource based, from individually to population based, and from spatially non-explicit to explicit. However, most of them assume that soil resources are spatially homogeneous within the plant neighborhood, a condition that is not often found in nature.

Spatial heterogeneity of belowground resources is ubiquitous within natural plant communities. Nitrogen, phosphorus and other key nutrients exhibit heterogeneity at scales less than a meter in a variety of community types including sagebrush steppe (Jackson & Caldwell, 1993a,b), deciduous woodland (Farley & Fitter, 1999), upland hardwood forest (Gross et al., 1995), desert (Schlesinger et al., 1996), tropical dry forest (Gonzalez & Zak, 1994), and warm-temperate conifer forest (Lister et al., 2000). Because growth of individual plants can be influenced strongly by soil heterogeneity (Einsmann et al., 1999; Hutchings et al., 2000), it follows that interspecific competition should be affected as well.

Root foraging behavior of individual species may influence competitive ability in heterogeneous environments. A key behavior is morphological plasticity, which is an adjustment in root system structure in response to changing environmental conditions. Two major changes in structure have been documented: precision and profusion. Precision is defined as root proliferation in nutrient rich microsites (Campbell et al., 1991, Fransen et al., 1998; Einsmann et al., 1999; Farley & Fitter, 1999; Robinson et al., 1999). Profusion is the plant's ability to produce an extensive root system, which may enhance its capacity to locate nutrients (defined as scale in Campbell et al., 1991; Einsmann et al., 1999). Species vary considerably in these two behaviors (Campbell et al., 1991;

Einsmann et al., 1999); however, the extent that either behavior contributes to competitive ability in heterogeneous soil is not known.

In addition to root foraging behavior, total plant biomass response to heterogeneity may predict competitive ability. Plants that have greater biomass under patchy than under homogeneous nutrient conditions have been described as "sensitive" (Wijesinghe & Hutchings 1997; Einsmann, et al.1999). When assessed using monocultures, sensitivity is also a measure of the impact of heterogeneity on intraspecific competition. Sensitivity may or may not be related to morphological plasticity in the root system (Einsmann et al., 1999).

Understanding how roots respond to nutrient patches is a growing interest among ecologists. However, many studies have dealt with single-plant, or monoculture responses to nutrient heterogeneity, rather than mixed community responses, which typify natural environments. Results from this previous work are conflicting. Single plants may be quite sensitive to nutrient heterogeneity (Einsmann et al., 1999) whereas mean response in monocultures may not (Casper & Cahill, 1996; Casper et al., 2000). This study explores effects of nutrient heterogeneity on plant response and on intra- and interspecific competitive outcomes for six plant species grown in garden plots. Further, we determine if profusion and precision are correlated with competitive ability (sensitivity) in spatially heterogeneous soils. According to our previous research using individual plants and monocultures, these species vary in precision, profusion, and sensitivity, but relationships among these traits are unclear (Mou et al., 1995, 1997; Einsmann et al., 1999). Here we hypothesize that some species will be sensitive to heterogeneity, and that the degree of sensitivity will be related to either precise foraging or rapid growth (i.e., profusion). Furthermore, we predict that heterogeneous nutrient conditions can shift interspecific competitive interactions by enhancing the relative growth of either precise foragers, or rapidly growing species.

METHODS

Species and study site

We chose six early to mid successional species that co occur in warm-temperate, coastal plain forests of the southeastern USA. Three are annuals [*Chamaecrista nictitans* (L.) Moench, *Hypericum gentianoides* L., *Erechtites hieracifolia* (L.) Raf.], one is a perennial (*Solidago altissima* L.), and two are trees (*Pinus taeda* L. and *Liquidambar styraciflua* L.). During the first year of growth after seed germination, *E. hieracifolia* and *S. altissima* grow rapidly to one or more meters in height, *H. gentianoides* and *C. nictitans* develop into shorter (about 0.5 M tall), dense, shrub-like forms, and the two trees (*L. styraciflua* and *P. taeda*) produce relatively shorter stems and narrower crowns. In October 1997, seeds of the four herbaceous species were collected from early successional plant communities at Savannah River Site, Aiken and Barnwell counties, South Carolina, USA. Improved tree seeds were obtained from Flint Nursery in Byromville, Georgia.

The study was conducted at the Joseph W. Jones Ecological Research Center at Ichauway, which is located in the gulf coastal plain of Georgia (Baker County). In January 1998, four 15 x 20 m blocks were established in an old agricultural field. The study was blocked to control for trends in soil conditions due to a limestone outcrop in the center of the field. Soils were Lakeland Series (sandy, thermic, coated, Typic Quartzipsamments). The site had previously been used for agriculture and contained rye (*Secale cereale* L.). The area had lain fallow for several years. After the blocks were disked to a depth of 20 cm and rolled to repack the soil, all vegetation and seeds were killed by fumigation with methyl bromide (98%) and chloropicrin (2%).

Plant preparation and establishment

From January 12 until March 1 seeds were geminated in a greenhouse at Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. When plants had attained sufficient size to survive transplant (4 cm height, 2 leaves),

seedlings were planted into plugs (2.5 x 2.5 wide x 6.3 cm deep) in a 50:50 (by volume) mixture of Metro Mix 200 (Scott's Sierra Horticulture Products, Marysville, OH) and sterilized sand. To inoculate for native microbes, 1.5 g of soil from the Georgia field site was added to each plug. Plants were watered twice daily, ensuring that the growing medium was kept near field capacity. Plants were fertilized twice in March (2, 16) with a liquid 20-20-20 fertilizer solution (100 ppm).

In late February, square plots (90 x 90 cm and 120 x 120 cm) were established for intraspecific and interspecific competition treatments, and for the mixed community experiment (Table 1, Fig 1). During March 26-28, seedlings were transported to the Georgia site, and planted within plots using 15 cm spacing. Cylindrical plugs of soil (8.5 cm deep by 2.5 cm width) were removed from the plots using a sink tube to make uniform holes; and plants were placed within the holes. Soil was gently compacted around the plants and any surplus soil from the extraction was discarded outside of the plot.

Over the course of the experiment less than 3 cm of rain fell. From May until mid July, 25-30 liters of water were applied by hand to each plot (depending on plot size). Plants were watered every third day to minimize drought effects. In spite of this watering regimen, *E. hieracifolia* appeared drought stressed, and it flowered and began senescing much earlier than expected. Therefore, the entire experiment was harvested July 15-17 after 3.5 months of growth.

Nutrient treatments

Nutrients were distributed in two arrays: homogeneous and heterogeneous. Each provided a mean of 2.1 grams of Osmocote slow release fertilizer (17-9-12 plus minors, Scott's Sierra Horticulture Products, Marysville, OH) per m^2. The nutrient release rate from this quantity of fertilizer is comparable to natural N mineralization rates found in nutrient poor, sandy, coastal plain soils (Burger & Pritchett, 1984; Bell and Binkley 1989). In homogeneous plots, fertilizer was tilled evenly into the surface 3 cm of soil across

Table 1.1 Size and configuration of garden plots used to test nutrient heterogeneity influences on competition

Competition Type	# target plants	# matrix plants	Total # of plants	Plot Dimensions (cm)	Total number of plots
Intraspecific	9	40	49	90 x 90	48
Two species	9	72	81	120 x 120	48
Six species	36	64	100	150 x 150	8

Figure 1.1: Configurations for garden plots to test soil nutrient heterogeneity influences on competition. (A) shows an intraspecific competition plot. Nine target plants (T) are surrounded by border plants (X) of the same species. Interspecific competition plots (not shown), are similar, but each target plant is completely surrounded by a matrix of another species. (B) shows a 10 x 10 six-species plot consisting of an inner 6 x 6 area where species (1-6) are randomized by Latin Square design, and an outer matrix consisting of 2 rows of border plants (X) which were a random mixture of the six study species. All intra- and interspecific plots are treated with uniform nutrient distribution (homogeneous treatment, not shown), or with patches of fertilizer placed such that each plant was 10.8 cm from the closest patch (heterogeneous treatment, open circles in B).

A.

X X X X X X X

X X X X X X X

X X T T T X X

X X T T T X X

X X T T T X X

X X X X X X X

X X X X X X X

B.

X X X X X X X X X X
O O O O O
X X X X X X X X X X

X X 1 3 2 5 6 4 X X
O O O O O
X X 3 2 4 6 1 5 X X

X X 5 6 1 2 4 3 X X
O O O O O
X X 4 5 6 3 2 1 X X

X X 6 4 5 1 3 2 X X
O O O O O
X X 2 1 3 4 5 6 X X

X X X X X X X X X X
O O O O O
X X X X X X X X X X

the whole plot including a 15 cm buffer outside the plot. In heterogeneous treatments the same total fertilizer was mixed into the top 3 cm of selected 5 x 5 cm patches representing ~1 % of surface area. Each plant was 10.8 cm away from the center of one nutrient patch (Fig 1).

Monoculture and two-species plots

Two monoculture plots (one with heterogeneous the other with homogeneous nutrient treatment) were established in each block. In each monoculture plot, the center nine plants were designated as target plants (Table 1, Fig. 1). Two-species plots were established for the following comparisons: annual x annual (*E. hieracifolia* x *C. nictitans*), perennial x tree (*S. altissima* x *P. taeda*), and annual x tree (*E. hieracifolia* x *P. taeda*). In each of these comparisons, target plants were surrounded by a neighborhood of matrix plants (Table 1, Fig 1). Both species were targets in half of the plots and used as the matrix species in the others, for a total of four plots per block (i.e., target and matrix x two nutrient treatments).

After harvest, oven dry biomass (60 °C) of the entire shoot was determined for each target plant, and for all matrix plants in the plot combined. In monoculture plots only, three root cores (10.2 cm width x 20 cm deep) were harvested with a bucket auger from random locations in homogeneous plots, and then pooled. Three cores each were also collected and pooled from randomly selected nutrient rich and nutrient poor locations within heterogeneous plots. The pooled samples were rinsed through a 2 mm mesh screen, and then roots were picked out from the remaining soil and debris. Roots were dried at 60 °C for 72 hours and then weighed.

Six species plots

In this experiment, a double border of plants surrounded an inner six by six grid containing the six species arranged in a Latin Square Design (Table 1, Fig 1). Each block included two such plots corresponding to the two nutrient

treatments. At harvest, aboveground portions of the inner 36 plants were collected and then dried to determine total oven dry biomass for each species (all plants of each species pooled).

Soil conditions and leaf area

Soil conditions were measured periodically to assess resource heterogeneity. One pair of 20 cm long stainless steel rods was placed in every plot (in a 15 cm wide unplanted buffer region surrounding the seedlings) for TDR (time domain reflectrometry) soil moisture analysis (technique from Topp *et al.*, 1980). Soil moisture readings were recorded for nine days, which were chosen to include three watering cycles. Soil moisture was measured after watering on the day that watering occurred, and for the next two consecutive days until the next watering, thus measuring the rapidity of soil drying prior to the next watering event. Soil temperature at 10 cm depth was measured with a soil thermometer at all plots on six consecutive dates (May 29-June 3).

Six weeks after plot establishment, anion/cation exchange membranes (3.8 x 3.8 cm) were inserted into the top 5 cm of soil in all planted monoculture plots, and in 4 unplanted, unfertilized plots. Nutrient adsorption by the membrane is an index of nutrient availability to the plant roots, and measures in unplanted plots provide an index of nutrient availability in the natural system, in the absence of plant uptake (Abrams & Jarrell, 1992). Membranes were left in the plots for 72 hours, removed and extracted with 25 ml (per membrane) of 2.0 M KCl and the extract was analyzed by LACHAT QuickChem AE autoanalyzer for nitrate and ammonium concentrations (Zwellinger Analytics, Inc Milwaukee, WI).
Leaf area was estimated using a Li-Cor LAI-2000 leaf area index (Li-Cor, Lincoln, NE) at all monoculture plots on two occasions, once six weeks after seedlings were established and again two weeks prior to harvest. Total leaf area was assessed by measuring light that penetrated to ground level.

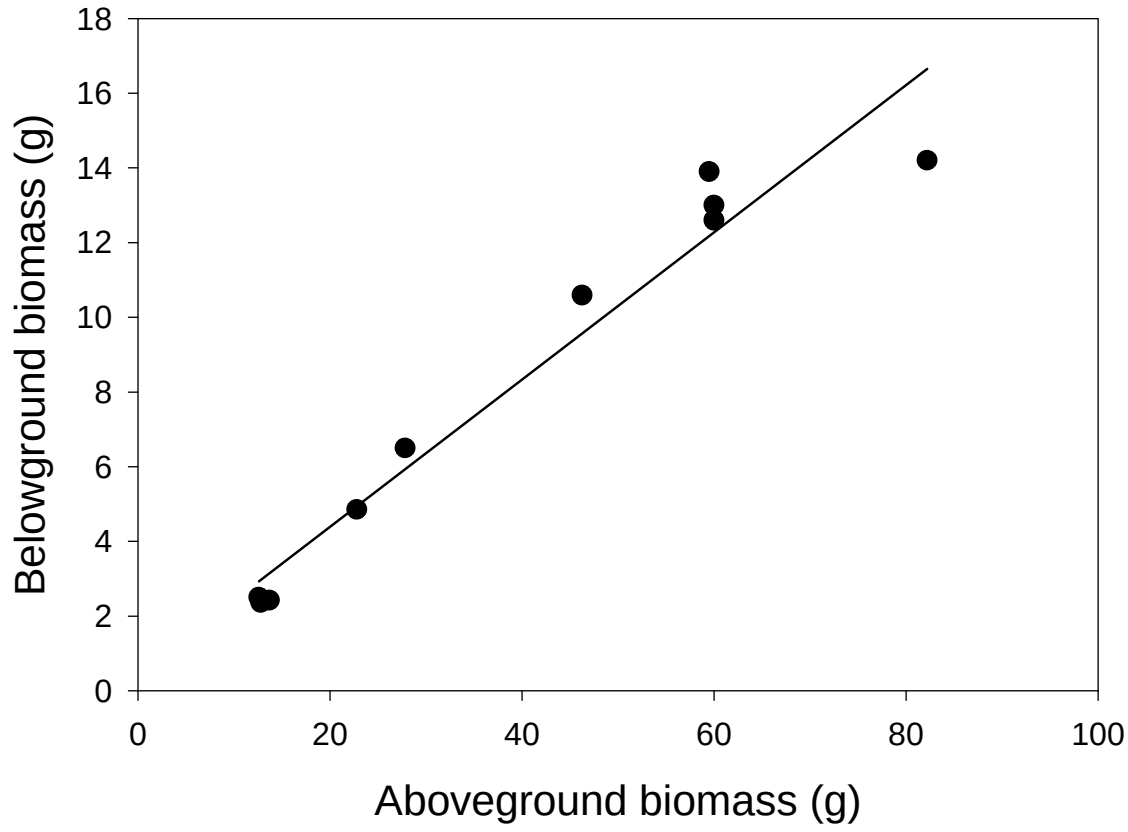
Analysis

Statistical Analysis System (SAS) software (SAS version 8.0, SAS Institute Inc., Cary, NC) was used for all statistical analyses. The experiment was a randomized complete block design, and therefore, a block effect was included in ANOVA where appropriate.

Precision of root foraging was determined using monoculture, heterogeneously fertilized plots only. In one analysis, the difference between root mass in high versus low fertility soil cores in a plot was divided by the total root mass collected from the plot. This calculation is a modification of relative fine root mass difference (RFRMD) that we have used in previous experiments to indicate precision (Mou et al, 1997; Einsmann et al, 1999). We conducted an ANOVA to test for species differences in RFRMD, and for each species, we also calculated confidence interval estimates for RFRMD, and inspected the intervals for overlap of zero (the expectation for no precision). A second analysis of precision included two factors, species and fertility patch within plots (high versus low). A split-plot ANOVA was used with these data to detect species and nutrient patch effects on root biomass. In this analysis, species were the whole plot factor and fertility treatment was the split plot factor.

Profusion and sensitivity analyses were conducted using aboveground biomass in monocultures. Differences among species in profusion (size of the root system) were assessed using a one-way ANOVA of aboveground biomass in homogeneous monoculture plots. We assumed that species with large aboveground biomass also had large belowground biomass, a trend we found by analyzing data from a previous study that used 5 of the 6 study species (Einsmann, 1999) in a potted plant experiment (Fig. 2). We also calculated profusion using leaf area index at final harvest in monoculture, homogeneous plots. To test for differences among species in sensitivity, biomass in heterogeneous monoculture plots was divided by biomass in the homogeneous monoculture (separate number for each block), and these ratios were analyzed

Figure 1.2 Relationship between mean aboveground and belowground biomass in five species (*C. nictitans, H. gentianoides, L. styraciflua, S. nemoralis, and P.taeda*) grown in pots with two fertility treatments (homogeneous and heterogeneous); n = 10 for each point. Data from Einsmann *et al.*, (1999). Regression is significant ($P < 0.001$, $r^2 = 0.95$).



using a one-way ANOVA. In this test, differences among species would indicate they differed in sensitivity to nutrient heterogeneity. We also evaluated sensitivity by using a two-way ANOVA to test for species, fertility treatment and species x fertility interaction effects (significant fertility effects in this analysis would indicate sensitivity). We calculated correlation coefficients between our indices of precision (RFRMD), profusion (aboveground biomass), and sensitivity (ratio of biomass in heterogeneous to homogenous plots) to determine if species show evidence for tradeoffs between traits.

Nutrient heterogeneity effects on interspecific competition were tested using a one-way ANOVA for each target and matrix plant combination. In each test, only target plant biomass was used as the response variable. The effects of spatial nutrient treatments on the six species communities were analyzed using Goldberg's competition index ($D = RY_{ix}-RY_{im}$), a measure of each plant species' competitive ability within the community (Goldberg, 1994). RY_{im} is the relative yield of species *i* in monoculture (calculated by the yield for a species in monoculture divided by the sum of the monocultural yields for all species). RY_{ix}, the relative yield of species *i* in mixture, is calculated similarly with the yield for a species in mixture divided by the sum of all mixture yields for all species. If D is zero, interspecific and intraspecific competition is equivalent for that species. One D value was calculated for each species in each nutrient treatment within each block. D values were analyzed using a two way ANOVA with species and nutrient treatment (heterogeneous versus homogeneous) as the main effects. We also calculated the absolute value of D for each species and summed these values for each plot. This is an overall index of community level change describing the effect of interspecific competition (Goldberg, 1994). A one-way ANOVA was used to test for the influence of nutrient arrangement on this sum of D index.

RESULTS

Soil conditions

Moisture in the top 20 cm of soil ranged from 13-33% across all plots on all sampling dates. The mean soil moisture was 20% at the driest point of the watering cycle, and 24% at the wettest point of the watering cycle. Moisture was uniform across fertility treatments (Table 2) and across all types of plots including monoculture, two-species and six-species plots (data not shown). Soil temperature averaged 32.2^{o} C, and no significant differences between nutrient treatments (Table 2) or between different types of plots were detected (data not shown).

Mean ammonium and nitrate values from high and low nutrient patches indicated that patches within heterogeneous plots were distinctly different (Table 2). Nutrient levels in the homogeneous treatment were intermediate, but were not significantly different from the nutrient poor patches in heterogeneous plots (Table 2).

General plant responses

Mean plant survival was high across species and throughout the experiment. Four weeks after initial transplant, survival was 99.2% and by the end of the experiment, overall survival was 97%. *Erechtites hieracifolia* had the highest mortality (11%). All other species had mortality less than 5%. Plant heights differed by species ($P < 0.0001$) but not by treatment. At harvest, *E. hieracifolia* was the tallest with a mean height of 54.0 cm, followed by *S. altissima* (43.7 cm), *H. gentianoides* (24.5 cm), *C. nictitans* (24.5 cm), *P. taeda* (17.5 cm), and *L. styraciflua* (15.4 cm). Leaf area index (m^2 foliage/ m^2 ground) was not significantly impacted by fertility treatment (Table 2), and as expected, leaf area increased between the middle and end of the experiment (ANOVA, $P < 0.05$).

Table 1.2 Soil measures and leaf area index measured in garden plots, showing contrasts between heterogeneous and homogeneous nutrient treatments and between high and low fertility patches within heterogeneously fertilized plots

Variable	# Plots	Measurement date or location	Heterogeneous		Homogeneous	
			Mean	SD	Mean	SD
Soil moisture (%)	104	mean of 9 dates	20.86[a]	3.80	21.43[a]	3.50
Soil temperature (^{o}C)	104	mean of 6 dates	32.15[a]	0.48	32.16[a]	0.49
Ammonium (ppm)	144	high fertility	85.99[a]	65.24	10.15[b]	11.76
		low fertility	5.17[b]	10.25		
Nitrate (ppm)	144	high fertility	59.40[a]	49.57	16.28[b]	4.99
		low fertility	4.99[b]	4.81		
Leaf Area Index (m^2	48	May 28-30	0.86[a]	0.52	0.97[a]	0.56
foliage/m^2 ground)		June 11-13	1.27[a]	0.54	1.42[a]	0.67

Within variables, measures with different superscripts are significantly different at $P < 0.05$.

Table 1.3 Mean plant responses measured in monoculture plots

	Precision		Profusion				Sensitivity	
Species	RFRMD	Rank	Aboveground biomass (g)	Biomass rank	Leaf Area Index (LAI)	LAI rank	Biomass ratio[3]	Rank
S. altissima	0.419[a]	1	8.25[a]	1	2.05[a]	1	0.97[a]	4
L. styraciflua	0.322[a]	2	1.41[c]	5	1.12[ab]	4	0.92[a]	5
C. nictitans	0.220[a]	3	4.66[b]	4	1.90[a]	2	0.98[a]	3
P. taeda	0.046[a]	4	1.31[c]	6	0.58[b]	6	1.11[a]	1
E. hieracifolia	-0.003[a]	5	6.73[ab]	2	1.72[a]	3	0.91[a]	6
H. gentianoides	-0.017[a]	6	4.82[b]	3	1.08[ab]	5	1.02[a]	2

RFRMD = (root mass in high fertility – root mass in low fertility cores) / (sum of mass in both cores); $CI_{95\%}$ RFRMD for *S. altissima* > 0. Sensitivity is aboveground biomass in heterogeneous plot within a block divided by biomass in homogeneous plot in same block; Within a column, superscript letters that are different indicate significance (ANOVA followed by the REGW Multiple Range Test, $P < 0.05$).

Precision, profusion and sensitivity

According to comparisons of confidence interval estimates, only *S. altissima* had a RFRMD greater than zero (Table 3), an indication that this species was a precise forager. The split-plot ANOVA of species and soil fertility effects, however, detected significant species (df = 5,15; $p = 0.004$), fertility (df = 1,18; $p = 0.004$), and species x fertility interactions (df = 5,18 $P < 0.02$; Fig 3). Thus, at least some species were precise (indicated by the significant fertility treatment effect) and species differed in the degree of precision (significant species x fertility effect). According to RFRMD values, the most precise species were *S. altissima* and *L. styraciflua*, and the least precise were *E. hieracifolia* and *H. gentianoides* (Table 3).

Species differed significantly in profusion. Based on both aboveground biomass and leaf area, *Solidago altissima* ranked first (largest) in profusion and *P. taeda* ranked last (Table 3). Ranks for the remaining species varied according to measure used, but trees were always small relative to herbs (Table 3).

No species were sensitive according to our one-way and two-way AVOVAs ($P > 0.25$). Mean ratios of biomass in heterogeneous/ homogeneous plots, our index of sensitivity, had a narrow range of only 0.92 to 1.11 (Table 3). When analyzed across species, correlations were weak between precision and profusion (r = 0.17), precision and sensitivity (r = -0.34), and profusion and sensitivity (r = -0.40).

Competition in two-species plots

The spatial arrangement of nutrients had a significant effect on competitive interactions in two of the two-species mixtures. Both *S. altissima* and *P. taeda* target plants grew larger in the heterogeneous treatment than in the homogeneous treatment (ANOVA; df = 1,3; $P < 0.05$). Precision was more related to these results than was profusion or sensitivity. In both cases, the target species was more precise than the matrix species. Furthermore, in three of the remaining four cases tested (all but *E. hieracifolia* grown as a target with *P.*

Figure 1.3 Root dry mass in soil cores collected from high and low fertility patches in monoculture listed left to right in descending RFRMD: Sa = *Solidago altissima*, Ls = *Liquidambar styraciflua*, Cn = *Chamaecrista nictitans*, Pt = *Pinus taeda*, Eh = *Erechtites hieracifolia*, Hg = *Hypericum gentianoides*. Error bars are standard errors based on n = 4 blocks.

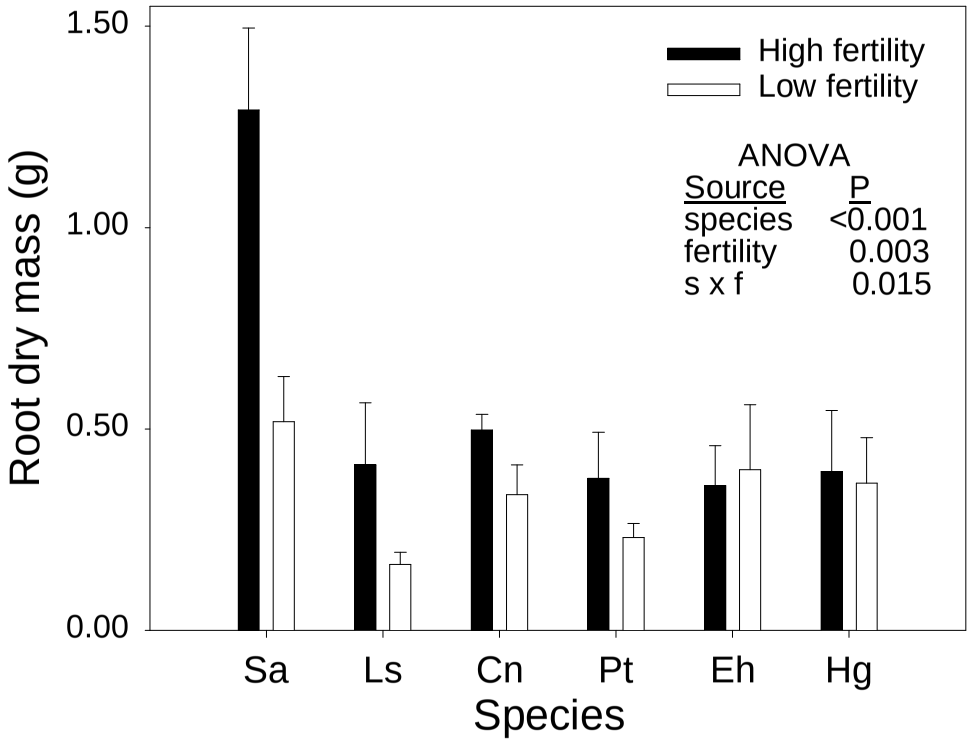
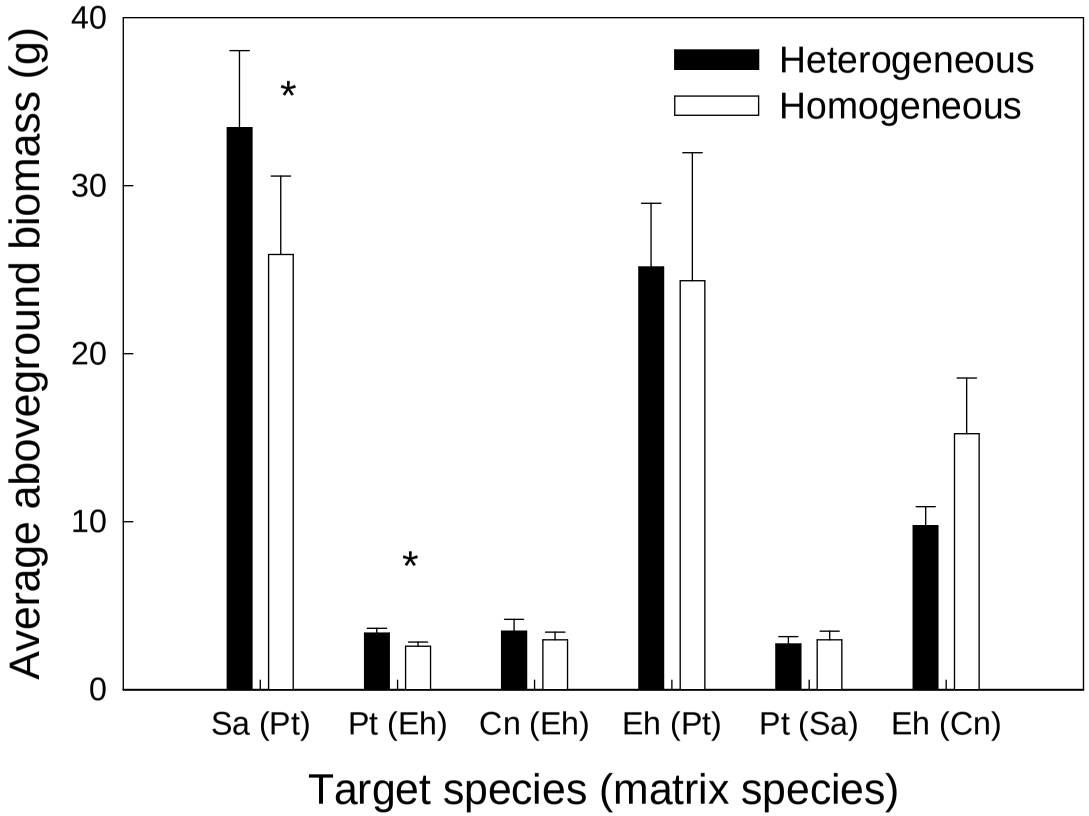


Figure 1.4 Aboveground biomass for target plants in competition with a matrix species. Notation is target species (matrix species). Error bars are standard errors based on n = 4 blocks. Asterisk (*) indicates a significant difference in biomass between heterogeneous and homogeneous treatments. Sa = *Solidago altissima*, Ls = *Liquidambar styraciflua*, Cn = *Chamaecrista nictitans*, Pt = *Pinus taeda*, Eh = *Erechtites hieracifolia*, Hg = *Hypericum gentainoides.*



taeda), although no significant heterogeneity effects were found, the overall trend was that more precise foragers had larger biomass in the heterogeneous treatment, while less precise foragers tended to have a larger biomass in the homogeneous treatment (Fig 4). When these results were compared with profusion ratings (Table 3), the two significant cases conflicted (i.e., the larger profuse plant gained by heterogeneity in the *S. altissima* (*P. taeda*) pair, but the opposite occurred for the *P. taeda* (*E. hieracifolia*) comparison). Furthermore, in the remaining four tests, the larger species gained biomass in two cases, and lost biomass in the other two cases. Finally, the two species with relatively low sensitivity rank in monoculture (*S. altissima* and *E. hieracifolia*) were at opposite ends of the spectrum in terms of response to heterogeneity in two-species mixtures.

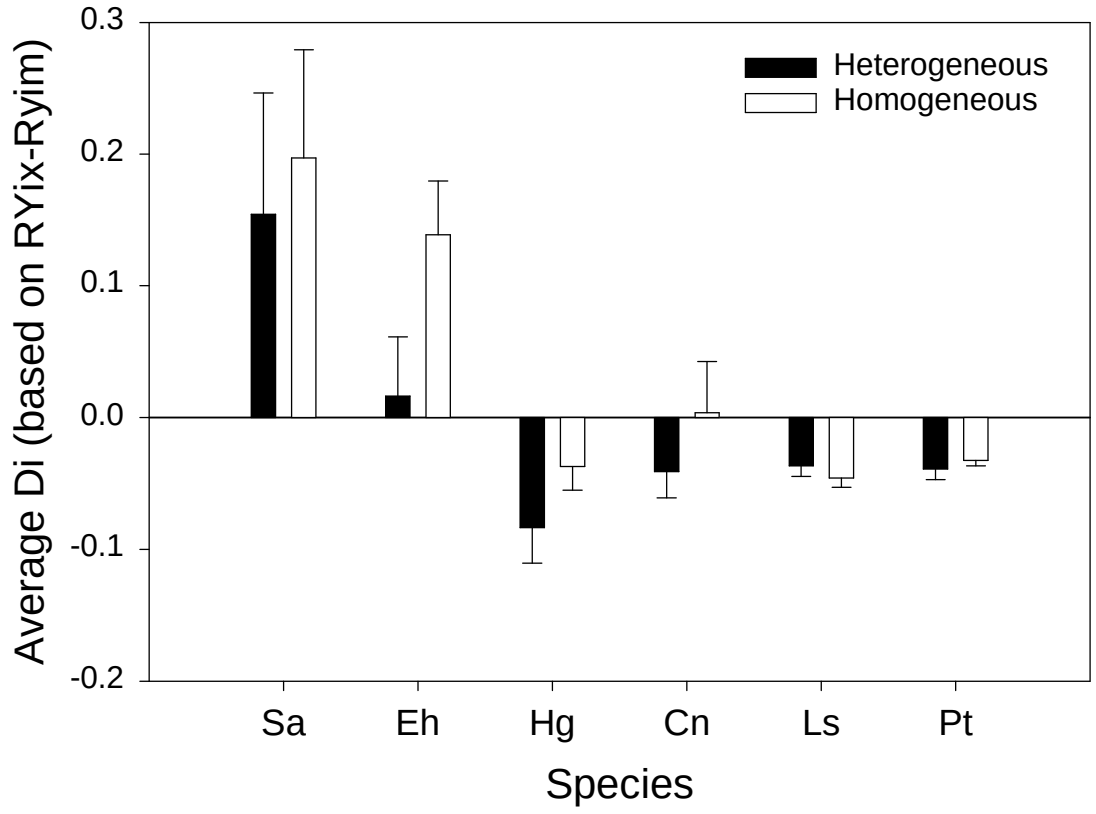
Competition in six species plots

In six-species plots, species differed in interspecific competitive ability as measured by Goldberg's D index (ANOVA; df = 5,31; $P < 0.001$). However there were no heterogeneity treatment effects (df = 1,31; $p = 0.379$), nor were there species x treatment interaction effects (df = 5,31; $p = 0.928$). The two largest species (i.e., with profuse roots systems) performed better in mixture than predicted based on monoculture yields (Fig. 5). Therefore, mean D values calculated for each species (heterogeneous and homogeneous plots combined) were positively correlated with profusion (r = 0.79). They also had a positive correlation with precision (r = 0.48) and a negative one with sensitivity (r = -0.36). Heterogeneity treatments had no significant impact on the sum of D values within plots, an index of mean community change (df = 1,3; $p = 0.765$; D for homogeneous = 0.406 (se = 0.092) and for heterogeneous = 0.362 (0.063).

DISCUSSION

Our first hypothesis, that some species will be sensitive to heterogeneity and that the degree of sensitivity will be related to precision or profusion of root foraging, was not supported. No significant sensitivity effect was detected in any

Figure 1.5 Goldberg's D_i index (relative yield of plants in mixture minus relative yield in monoculture) with species listed in order of decreasing profusion (left to right). Sa = *Solidago altissima*, Eh = *Erechtites hieracifolia*, Hg = *Hypericum gentianoides*, Cn = *Chamaecrista nictitans*, Ls = *Liquidambar styraciflua*, Pt = *Pinus taeda*. Error bars are standard errors based on n = 4 blocks. Nutrient arrangement was not significant (ANOVA, $P > 0.05$).



species, and our index of sensitivity had a very small spread around the expected value for no sensitivity (i.e., 1.0; Table 3). Furthermore, the index of sensitivity was only weakly correlated with profusion and precision. Our results, therefore, are consistent with other studies that have shown little or no effect of soil nutrient heterogeneity on mean biomass in multi-plant monocultures (Casper et al., 2000). The monocultures did show, however, that plants responded to nutrient heterogeneity with proliferation in patches (precision), and that the degree of proliferation differed among species. Other studies have also shown interspecific differences in degree of precision (Caldwell 1991; Mou et al.,1995; Einsmann et al., 1999). Differences in precision in our study however, were not as striking as in an earlier greenhouse study of the same species using a single plant per pot (Einsmann et al., 1999). This suggests that the presence of conspecific neighbors may decrease root foraging precision.

Our second hypothesis, that under heterogeneous nutrient conditions interspecific competitive interactions would shift so that either precise foragers or rapidly growing (profuse) species would benefit, was partially supported. Success in a competitive environment was determined by average aboveground biomass in heterogeneous treatment compared with aboveground biomass in homogeneous treatment. In two-species plots, two (of three) cases showed the more precise species of the pair gained a significant competitive advantage (increased aboveground biomass). In the remaining test, the same trend in means was observed although the results were not statistically significant. In pairs where the target was profuse (less precise than the matrix species), biomass tended to be higher in the homogeneous treatment (in two of three cases), although the results were not statistically significant. Thus, in some species pairs, target plant response was altered by spatial heterogeneity of soil nutrients.

In the six species experiment, no significant nutrient distribution effects were found. Relative growth rate contributed disproportionately to overall community response, while heterogeneity (treatment) effects were dwarfed by comparison. In fact, a species' interspecific competitive ability (D) was more

strongly related to profusion than to any other factor we measured. The lack of any soil nutrient arrangement effects was surprising, since heterogeneity influenced competition in the two species plots.

Why were effects of belowground root foraging evident in two-species plots, and unimportant in intraspecific competition (monoculture) and six-species (mixed community) plots? First, we speculate that root foraging may be less important in monocultures than in mixed-species plots. In single-species plots, an individual plant that proliferates in rich patches may not gain biomass because its neighbors employ the same strategy, thus offsetting any possible advantage. According to Casper et al. (2000), roots of neighboring plants that proliferate in patches reduce nutrient availability in the patch so that it is similar to background nutrient levels. Second, results of the study were likely confounded by unintended variation in size asymmetry and density of individual species; two factors that influence competitive interactions (Rees et al., 1996). Although all plots had the same total density (15 x 15 cm spacing, or 44 plants m^{-2}), size asymmetry increased, and density for individual species decreased as more species were added to a plot. Target plant density for any one species was 44 m^{-2} in monocultures, 16 m^{-2} in two-species plots, and only 6 m^{-2} in six-species plots. For some of the larger species, the low density of conspecific neighbors, and the likelihood that neighbor plants were smaller may have led to weaker competition in the six species plots than in either the monocultures or two-species plots. Even when plots with the same number of species are compared, the larger size of fast growing herbaceous species created more intense competitive pressure than occurred in plots dominated by trees. Thus, caution must be taken in interpreting results of our experiments, and new work is needed to tease out the independent influences of species, density, and intra- versus interspecific effects.

Our data provide direct support for existence of morphological plasticity, and for possible shifts in competitive interactions as a result. There is indirect evidence that foraging mechanisms other than precision may operate to enhance uptake in patchy environments. One species, *P. taeda* had greater aboveground

biomass in the heterogeneous treatment when grown in competition with *E. hieracifolia*; however it was not very precise, even though it was more so than *E. hieracifolia*. Physiological plasticity might have provided *P. taeda* with an advantage in patchy soil. Grime et al. (1986) argued that physiological plasticity should be more common in unproductive environments, while morphological plasticity should predominate in productive environments. It follows that coastal plain species (and other species from relatively unproductive environments) need to be examined for other root foraging traits including physiological (uptake) and demographic plasticity (turnover). In addition to physiological plasticity, there are many other facets of nutrient foraging that were not addressed by this study, yet are potentially important for explaining our results. These include within-species genetic variability, root-shoot signaling, mycorrhizal symbioses, and soil fauna (Zhang & Ford, 1998; Beveridge, 2000).

Despite implementation of a regimented irrigation plan, drought effects likely played a substantial role in this experiment. Although soil moisture in the top 20 cm of soil was generally adequate, we suspect that deeper soil layers and soils adjacent to the plots were very dry, thus limiting available volume of soil that plants could exploit. Due to lack of natural rainfall, plants matured early and were harvested earlier than expected. Plants were probably responding to nutrient treatment, competition and drought stress. Although nutrient and competition levels were contrived, drought stress was unintended. Our moisture data show that moisture levels were consistent across species and treatments. However, individual species can and do exhibit different tolerance levels to moisture stress. *E. hieracifolia* appeared to be the least drought tolerant species because it had the greatest mortality rate of individual leaves and whole plants, and had unusually early flowering.

Insufficient sample size in our study precluded a powerful test of relationships between root foraging and other plant traits. However, as we have seen in another study (Einsmann et al., 1999), precision of foraging appeared unrelated to either growth form or profusion. Within the annual plants, for

example, *C. nictitans* showed evidence of precise foraging whereas *E. hieracifolia* did not. Only a weak positive correlation ($r = 0.17$) between precision and profusion was found. In contrast, a study by Campbell et al. (1991) documented an inverse relationship between profusion and precision for eight herbaceous species.

In this paper we have shown that individual plant responses to patchy resources can influence competitive interactions between neighboring species. Although our data suggest that morphological plasticity of root systems may be related to competitive ability, it remains to be determined how important this root foraging characteristic is relative to others. Finding the cause for heterogeneity effects will be difficult because plants may use different types of plasticity (morphological, physiological, demographic) in concert in order to best exploit a particular set of environmental conditions. Shifts in the predominant foraging mechanism may occur as a result of abiotic or biotic factors. Furthermore, the characteristic complexity of the rhizosphere (soil fauna, mycorrhizae, bacteria) is enhanced by soil nutrient heterogeneity.

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Chapter two: Root Foraging Characteristics: Morphological versus Physiological Plasticity

ABSTRACT

A split root pot system was used to examine root foraging response to nutrient heterogeneity in four coastal plain species native to Georgia. Morphological and physiological plasticity were quantified under two nutrient treatments (homogeneous and heterogeneous). Each treatment contained the same overall fertility level; however, in the heterogeneous treatment, fertilizer was concentrated in only one half of the pot. Root morphological plasticity (precision) was measured by a ratio (RFRMD) comparing root biomass in high versus low fertility pot halves. Root physiological plasticity was estimated by measures of ^{15}N uptake per unit root mass after dosing plants for 48 hours with a weak concentration of ^{15}N labeled ammonium nitrate. Total ^{15}N uptake was used to measure the combined effects of morphological and physiological plasticity. Nutrient treatment had no effect on aboveground biomass, however, total root mass was significantly greater in the heterogeneous treatment ($p = 0.0001$), and all species had significantly greater RFRMD in heterogeneously fertilized pots ($p < 0.001$), indicating that all of them were precise. Physiological plasticity (uptake rate of ^{15}N per unit root mass) was greater in the low fertility half of heterogeneous pots for all species. However, the magnitude of increase differed among species, creating a significant species by fertility interaction term ($p = 0.04$). Overall uptake of ^{15}N was not significantly influenced by nutrient treatment. We conclude that all four species used a combination of morphological and physiological plasticity to enhance nitrogen uptake. Compared to plant roots in high fertility patches, ^{15}N treated plant roots in low fertility patches were able to take up comparable amounts of nitrogen. Therefore, physiological plasticity was important in our ^{15}N study - which used relatively short duration patches of ^{15}N. Morphological plasticity may play a greater role

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when nutrient patches are more stable in time, evidenced by root proliferative response to patches of slow release fertilizer.

KEYWORDS: nutrient heterogeneity, coastal plain species, ^{15}N uptake, root foraging behavior, root plasticity

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INTRODUCTION

Within heterogeneous soils, plants use morphological and physiological plasticity to exploit nutrient rich microsites. Morphological plasticity, especially proliferation of roots in nutrient rich patches (precision) has been demonstrated in many species (eg., Campbell et al., 1991; Einsmann et al., 1999). Studies have shown that trees and herbs may differ in morphological plasticity (Einsmann et al., 1999; Mou et al., 1995; 1997), or show similar foraging strategies (Ludovici and Morris, 1996). Precision may lead to increased uptake of nutrients in patchy soils (Drew and Saker, 1975; Robinson et al., 1994; Cahill and Casper 1999; Hodge et al., 1999; Robinson et al., 1999), and so, influence competitive interactions (Caldwell et al., 1991; Jackson and Caldwell, 1996; Mou et al., 1997; Robinson et al., 1999), although other studies suggest that in a plant community, heterogeneity effects are diminutive or nonexistent (Casper and Jackson, 1997; Cahill and Casper, 1999).

Physiological plasticity, a change in uptake rate per unit of root surface has been shown for several species (Grime et al., 1986; Hutchings and deKroon 1994; Caldwell et al., 1996). However, plant roots may proliferate in conjunction with increased uptake (Jackson et al., 1990). Therefore, teasing out independent effects of morphological and physiological plasticity can be difficult. These processes may work cooperatively to help plants remove mobile and immobile nutrients from the soil matrix. Increased physiological plasticity may be especially important for mobile forms of nutrients like nitrate, while increased proliferation may be more important for exploiting immobile nutrients such as phosphorus (Caldwell et al., 1991). Most likely, for any one species, a combination of these two traits may account for plant responses to soil heterogeneity (Hutchings and deKroon, 1994).

What is the cost to the plant for these root foraging behaviors? For morphological plasticity, the main cost is the carbon investment of building root mass. For physiological plasticity, it may be the metabolic cost of up-regulating enzyme production to increase uptake. Thus a plant may face a tradeoff between

morphological and physiological plasticity. Using both mechanisms at the same time could be an inefficient use of carbon to obtain nutrients.

Historically, site fertility has been linked with root foraging behavior (Grime 1974; 1977). In productive environments, species adapted to fertile sites may have greater capacity for morphological plasticity (presumably enhanced resource acquisition), and in this case, morphological plasticity probably confers a selective advantage over species without morphological plasticity (Crick and Grime 1987). In unproductive environments, morphological plasticity is much more costly and may prove disadvantageous to plants (with costs in carbon and resources outweighing the benefits of root foraging, Crick and Grime 1987). If these hypotheses are correct, fast growing species should have a greater tendency for morphological than physiological plasticity, while slow growing species should show the opposite trend.

However, since the environment around a particular plant is not universally fertile or infertile, but instead a patchwork of fertile and infertile microsites, overall fertility levels may not be the key to successful root foraging strategies. Stability of the patch in time may be more important. An explanation of root foraging strategies that incorporates the temporal dimension of heterogeneity is essential. When patches are temporally stable, proliferation may be the best strategy because the plant will gain high levels of nutrient per unit of carbon investment. When patches are temporally unstable, roots will increase uptake (rather than root mass), thus maximizing uptake efficiency, while at the same time minimizing loss of carbon via less investment in root biomass.

This study evaluates the importance of two root foraging responses to nutrient heterogeneity: morphological and physiological plasticity. Our first hypothesis is that each of four test species will have both morphological (root proliferation in nutrient rich environment) and physiological plasticity (disproportionate increase in uptake per unit root in nutrient rich environment). Secondly, we hypothesize that there is a tradeoff between morphological and physiological plasticity, with rapidly growing plants exhibiting morphological

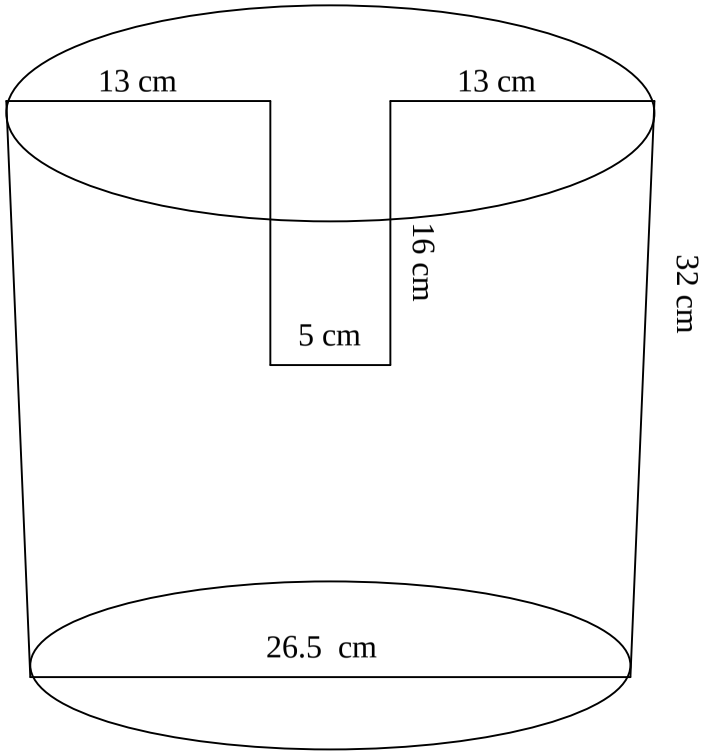
plasticity, and slowly growing plants exhibiting physiological plasticity. If this second hypothesis is rejected, then we propose that responses of plants to the temporal dimension of nutrient patches may be the controlling factor in determining root plasticity.

METHODS

A potted plant experiment was conducted in a glasshouse at Virginia Polytechnic Institute and State University, Blacksburg, VA from May - Oct 1999. Four species with different growth rates were selected: *Erechtites hieracifolia* (L.) Raf., *Solidago altissima* L., *Liquidambar styraciflua* L., and *Pinus taeda* L. The species are representative of early to mid-successional plant communities in coastal plain ecosystems and are an annual, a perennial and two trees respectively. In October 1998, seeds of the two herbaceous species were collected from early successional forests at Savannah River Site, Aiken and Barnwell counties, South Carolina, USA. Improved tree seeds were obtained from Flint Nursery in Byromville, Georgia.

From February 15 until March 25 seeds were geminated in a greenhouse. On May 4, when plants had attained sufficient size to survive transplant (4 cm height, 2 leaves), seedlings were planted into 12 inch diameter pots of coarse grade sand. All pots were equipped with plexiglass dividers to provide a physical separation of the pot halves (Fig 1). Dividers were constructed with a gap for plant taproots to form in the center (Fig 1). Pots were fertilized with 7.16 grams of Osmocote slow release fertilizer (NPK, 17-7-12 plus minors; Sierra Horticultural Products, Marysville OH) in two different treatment arrangements. In the homogeneous treatment, both halves of the pot received 3.58 g of fertilizer, while in the heterogeneous treatment, the enriched half of the heterogeneous treatment received nine times the concentration of the poor half (6.51 g and 0.65 g respectively). The total quantity of fertilizer was set to simulate nutrient mineralization conditions of upland coastal plain forests (Burger and Pritchett,

Figure 2.1 Side and aerial view of pot setup. Plexiglass dividers separate the two pot halves with a 4 inch depression to provide room for taproots. Nutrient arrangements are homogeneous (5x and 5x) and heterogeneous (x and 9x).



1984; Bell and Binkley, 1989) and to match a previous study of root foraging in similar species (Einsmann et al., 1999). In both homogeneous and heterogeneous treatments, the fertilizer pellets were mixed with the sand to a depth of 10 cm. Each pot was inoculated for microbes with 1 gram of sand from the native site (Savannah River Site, South Carolina).

Pots were kept moist by misting twice daily for the duration of the experiment. Plants were grown until roots reached pot edge (determined by periodic harvests of extra pots) and then, plants were harvested. Specific dates for harvest were: July 26-30 for herbaceous species (which grew relatively quickly) and November 26-29 for trees (which grew relatively slowly). To determine aboveground biomass, plants were clipped at ground level, basal stem diameter and height were measured, after which, plants were dried at 60 ^{o}C for 72 hours and weighed. Belowground biomass was determined for each half of each pot. Roots were separated from sand by washing them through a 2 mm mesh screen; then roots were dried and weighed.

Forty-eight hours prior to harvest, a subsample of pots was treated with 20 ml of 5 atom %, 100 millimolar dual labeled $^{15}NH_3$ $^{15}NO_3$ for a measure of physiological plasticity. Five randomly selected homogeneous pots, and ten randomly selected heterogeneous pots were treated. Either the high fertility half or the low fertility half of heterogeneous pots was dosed, giving five replicates per fertility level. Cylindrical plugs of soil (5.0 cm deep by 2.5 cm width) were treated with the ^{15}N labeled ammonium nitrate. A preliminary study with dye was used to determine the lateral and vertical diffusion bulb of the ammonium nitrate (data unpublished). After dosing with ^{15}N, plants were not watered until just prior to harvest. Root cores, slightly larger than the ^{15}N treated soil volume, but large enough to include the entire ^{15}N diffusion bulb, were removed. Root cores were harvested and processed as stated above. Aliquots of aboveground biomass, root biomass outside the cores, and root

mass in each core were homogenized by grinding with a Wiley Mill, followed by a Ball Mill grinder, and a subsample was weighed and analyzed by stable isotope ratio mass spectrometry (Stable Isotope Soil Biology Lab, Institute of Ecology, University of Georgia, Athens, GA 30602).

Degree of morphological plasticity was estimated using RFRMD (relative fine root mass difference) which was calculated as the difference in root mass between high and low fertility haves divided by total pot root mass; (high-low) / (high + low). In homogenous pots, RFRMD was calculated the same way, by using the two equally fertilized halves. Homogeneous pots are expected to have symmetrical root systems that should have a RFRMD of 0. Values different than zero in heterogeneous pots indicate morphological plasticity. Our RFRMD calculations are a modification of those we have used previously (Mou et al., 1995; Einsmann et al., 1999).

Physiological plasticity was measured by first calculating total ^{15}N taken up by the root and translocated to the shoot using the following formula: total ^{15}N = {(aboveground concentration in ^{15}N dosed plant – mean ^{15}N in non-dosed aboveground tissue) x total aboveground biomass in dosed plant} + {(non-core belowground concentration in ^{15}N dosed plant – mean ^{15}N in non-dosed belowground tissue) x the total non-core root mass}. We then calculated ^{15}N uptake per gram of root by: total ^{15}N /root mass in the dosed core. Differences in ^{15}N uptake per gram of root between nutrient rich patch dosed and those that had the nutrient poor patch dosed were used as an indication of physiological plasticity. In addition to root mass in the core, total fine root mass in the dosed half of the pot was used to provide a second estimate of uptake per gram of root. This estimate was used to offset possible variability in size and shape of the ^{15}N diffusion bulb. We also analyzed total ^{15}N in the shoot without regard to root mass. The total uptake of ^{15}N is an indicator of the joint

effects of morphological and physiological plasticity. In all analyses, fine roots were defined as < 2 mm in diameter. For both RFRMD (morphological plasticity) and ^{15}N uptake (physiological and morphological plasticity), two way ANOVA was used to determine species, fertility treatment, and species x fertility interaction effects. For an additional test of precision, estimates for RFRMD were calculated and examined to see if they overlapped 0. If the interval overlaps 0, there is no precision.

RESULTS

Fertility treatment had no effect on aboveground biomass according to a two-way ANOVA (Table 1). However, separate tests run for each species showed significantly greater aboveground mass in heterogeneous pots for *P. taeda* ($p = 0.038$). Belowground biomass was significantly greater in the heterogeneous than in the homogeneous treatment across all species (Fig 2). As a result of the root responses, effects of nutrient heterogeneity were also significant for total biomass (Table 1). Significant species effects were detected for both above and belowground biomass (Fig 2). Greatest shoot biomass occurred in *P. taeda* while the greatest root biomass occurred in *E. hieracifolia* (Fig 2).

All species had morphological plasticity. RFRMD (relative fine root mass difference) was significantly greater in the heterogeneous than in the homogeneous treatment in all species (Table 1, Fig 3). However, a separate test of the hypothesis that $RFRMD_{het} = 0$ using confidence interval estimates was rejected; zero fell within each interval.

Physiological plasticity was detected. Two-way ANOVA showed that nutrient and species effects are highly significant for both pergram root measures of ^{15}N uptake. Interaction effects were also detected (Table 1). All four species showed greater mean uptake in the low fertility half compared with the high fertility half of heterogeneous pots (Fig. 4). The

second measure of ^{15}N uptake produced very similar results (data not shown). When total ^{15}N values were analyzed, no significant nutrient or nutrient * species effects on total ^{15}N uptake were detected, although significant species differences occurred (Table 1). Since physiological plasticity was detected (Table 1, Fig. 4), the results in Fig 5 indicate that constancy of overall ^{15}N uptake was largely due to physiological plasticity. Greater root density (which occurred in high fertility patches) did not cause a corresponding increase in ^{15}N uptake.

DISCUSSION

Our first hypothesis that all four species display both morphological and physiological plasticity was supported. Root precision was present in all four species, although the degree of precision varied across species. Greatest precision, indicated by RFRMD values occurred in *E. hieracifolia* and weakest occurred in *S. altissima*. On the other hand, ^{15}N uptake per gram of root showed that there were differences in uptake rate related to spatial arrangement of fertilizer prior to the ^{15}N treatment (Table 1, Fig 4). All species had greater uptake in the low fertility half, and in most cases, the homogeneous treatment was intermediate. Greater uptake in the low fertility half is probably a function of two processes: increased inflow of nitrogen when root tissue is nitrogen starved (by diffusion), and active uptake by roots in nutrient poor conditions.

Our second hypothesis, that there is a tradeoff between morphological plasticity (exemplified by rapidly growing plants) and physiological plasticity (displayed by slowly growing plants) was not supported. Differences in morphological plasticity were detected; but they did not correlate with growth rate. The herbs grew more rapidly, but one herb had the greatest proliferation (*E. hieracifolia*) and the other had the weakest proliferation (*S. altissima*). The two trees were intermediate. Physiological plasticity, instead of varying strongly across species, was

Table 2.1: Results of ANOVA testing effects of fertility, species, and fertility * species interactions on plant responses, and the general hypotheses that each analysis was testing. * indicates significance at p = .05 level.

Response	Hypothesis Tested	Source	F-value	P	
Aboveground Biomass	-	Fertility	0.21	0.649	
		Species	2.94	0.038	*
		Fertility* Species	0.42	0.736	
Root biomass	-	Fertility	16.97	<0.001	*
		Species	7.77	<0.001	*
		Fertility * Species	0.50	0.684	
Total biomass	-	Fertility	5.00	0.028	*
		Species	1.47	0.229	
		Fertility * Species	0.12	0.945	
RFRMD	Morphological plasticity	Fertility	38.25	<0.001	*
		Species	0.32	0.808	
		Fertility * Species	2.08	0.101	
^{15}N/g root in Cores	Physiological plasticity	Fertility	3.83	0.062	
		Species	6.32	0.003	*
		Fertility * Species	3.24	0.040	*
^{15}N/g root in Pot half	Physiological plasticity	Fertility	3.72	0.066	
		Species	7.55	0.001	*
		Fertility * Species	2.82	0.060	*
Total ^{15}N Uptake	Morphological and plasticity	Fertility	2.01	0.186	
		Species	8.56	0.001	*
		Fertility * Species	1.66	0.151	

Figure 2.2: Dry mass at harvest for two herbs (*E. hieracifolia* and *S. altissima*) and two trees (*L. styraciflua* and *P. taeda*) grown in pots with nutrients distributed in patches or uniformly. Note, shoot and root biomasses have different scales.

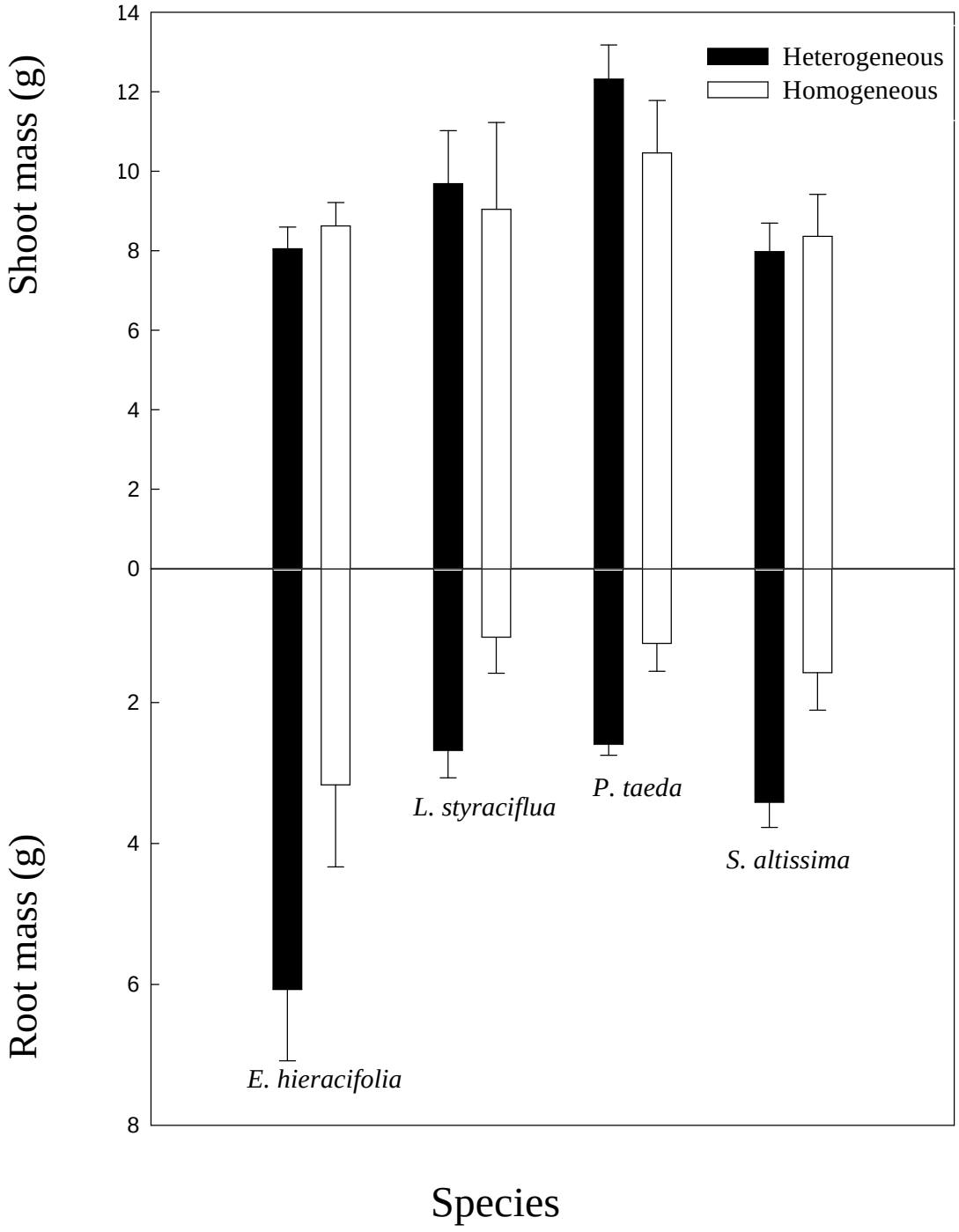


Figure 2.3: Precision of root foraging for four species grown under two nutrient treatments in pots. Precision was measured by relative fine root mass difference (RFRMD).

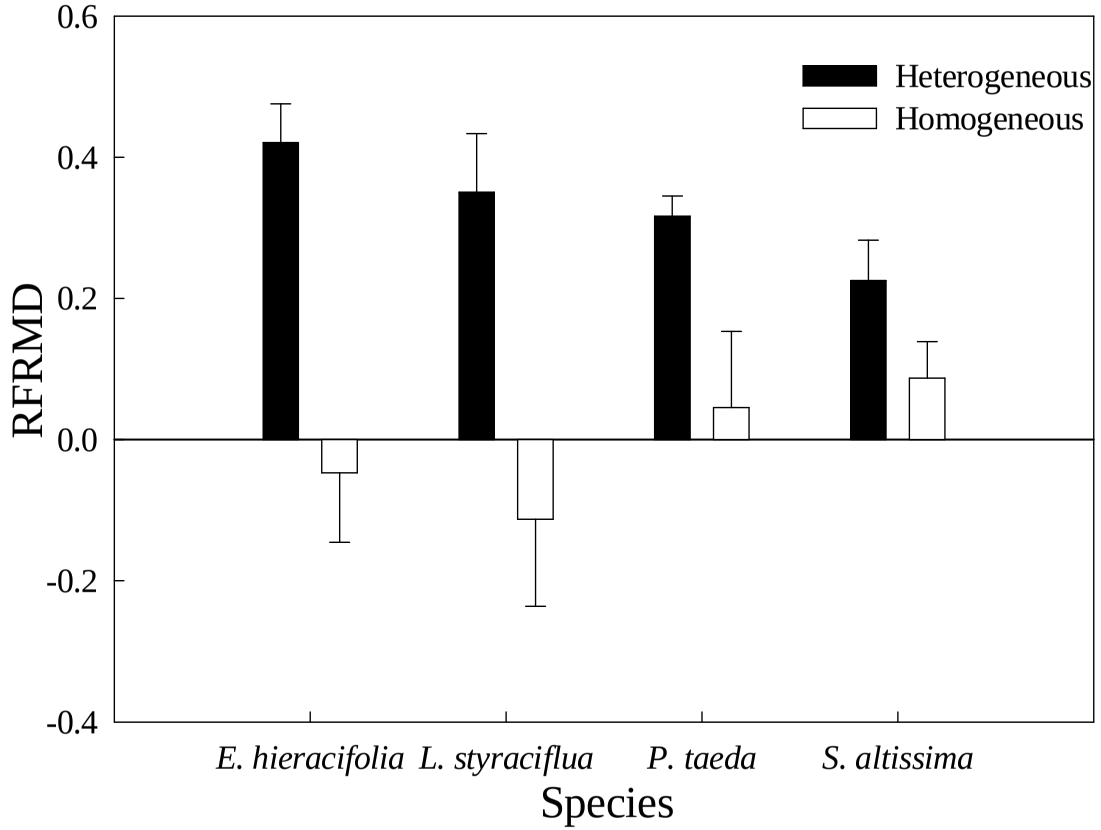


Figure 2.4: Uptake and translocation of ^{15}N (unadjusted for root mass) 48 hours after dosing roots with ^{15}N labeled ammonium nitrate. Abbreviations for species are: Erhi = *E. hieracifolia*, List = *L. styraciflua*, Pita = *P. taeda*, Soal = *S. altissima.*

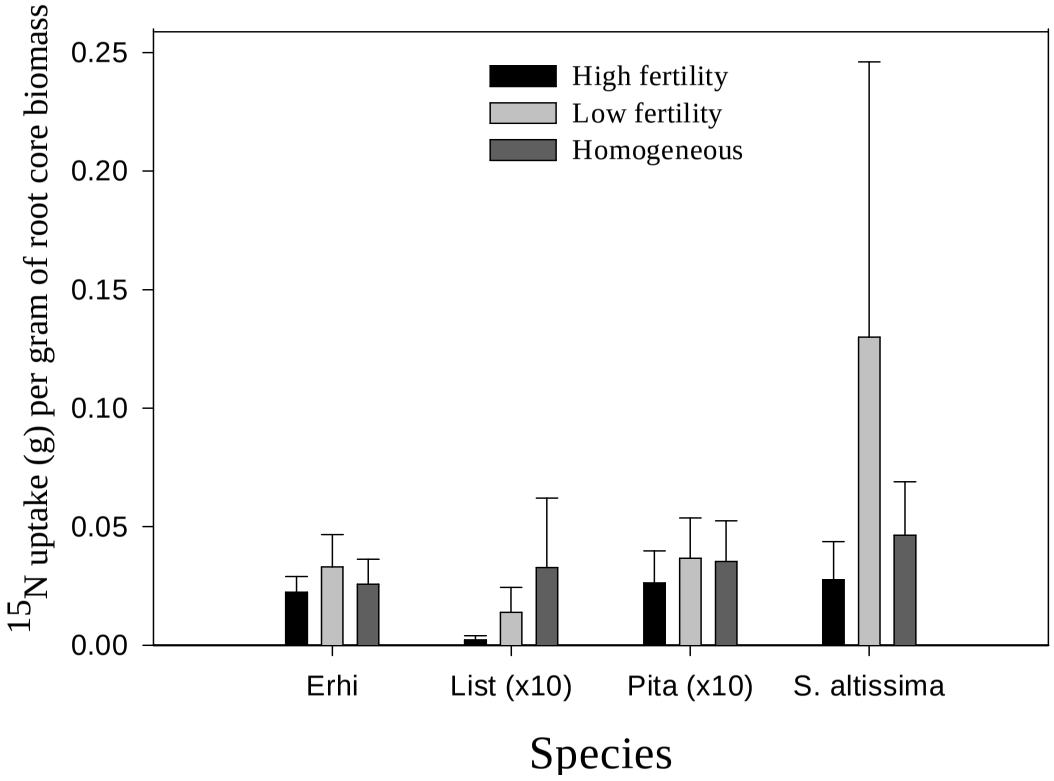
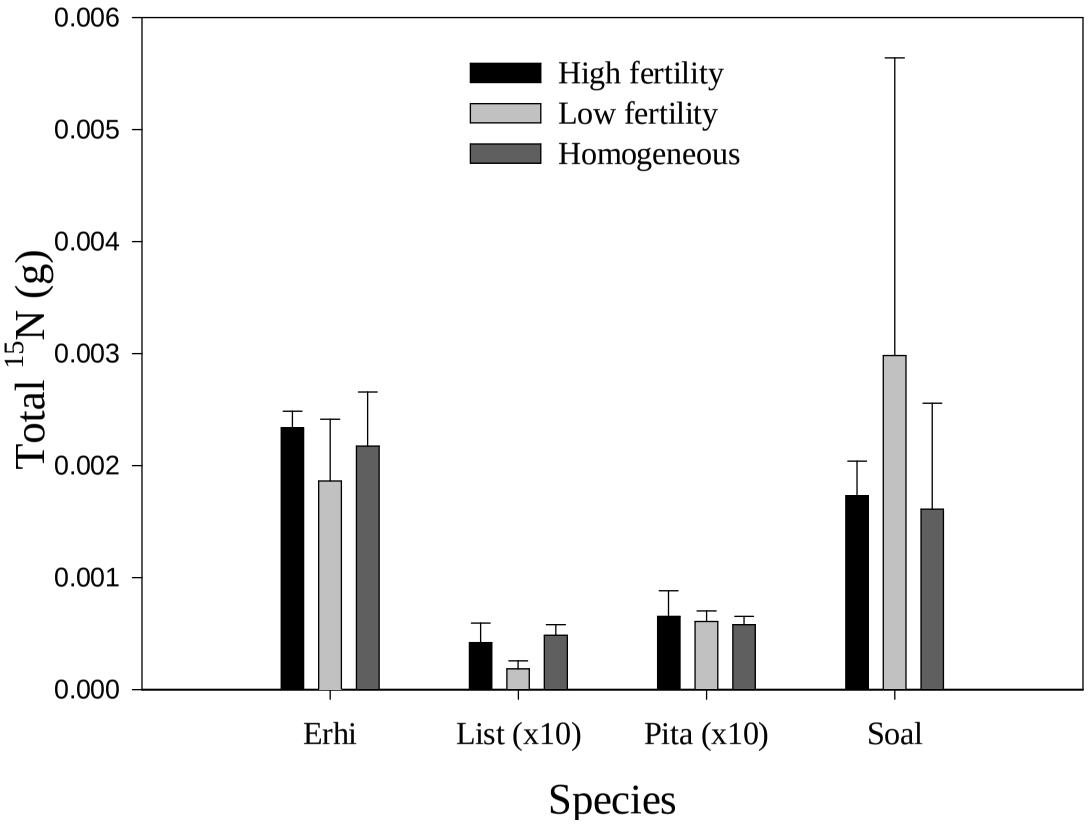


Figure 2.5 Uptake and transport of ^{15}N to shoot per gram of root, 48 hours after dosing with ^{15}N labeled ammonium nitrate. Figure shows uptake per gram of root tissue from the ^{15}N labeled soil core. Abbreviations for species are: Erhi = *E. hieracifolia*, List = *L. styraciflua*, Pita = *P. taeda*, Soal = *S. altissima*.



present in each one, and strongest differences occurred in one tree (*L. styraciflua*) and one herb (*S. altissima*). Thus, there was no tradeoff apparent between slow growing and fast growing plants in terms of physiology. However, since all plants had significant morphological and physiological plasticity, we conclude that a combination of these mechanisms is employed by plants, and that the mechanisms are not segregated with respect to growth rates.

We propose that the expression of morphological and physiological plasticity may be driven more by temporal dynamics of nutrient patches than by plant traits associated with fast or slow growth. This idea is consistent with proposed costs and benefits of each type of plasticity; i.e., in systems dominated by short nutrient pulses, physiological plasticity should be favored because new roots cannot be constructed rapidly enough to exploit ephemeral patches; and if so, roots will spend much of their life in nutrient poor conditions. In nutrient patches that are long-lived, proliferation may be a cost effective approach. Previous work by Eissenstat and Yanai (1997) indicates that roots respond to infertile sites by increasing longevity and that nutrient starved plants show increase uptake (Robinson et al., 1994). Bassirirad (2000) indicates that a high affinity transport system is implicated in controlling uptake of nitrogen in nutrient poor areas. However, none of these studies explain how pulses of nutrient versus long-lived patches affect uptake.

Alternatively, physiological plasticity may be favored if the most limiting nutrient is mobile (eg., nitrate; Grime et al., 1986; vanVuuren et al., 1996). Since it is unlikely that all sites within a community are dominated by just one patch type, or are limited by just one nutrient, it is logical that each species has the ability to employ morphological or physiological plasticity in response to local conditions. These arguments are supported by the documentation of both types of plasticity in species from a variety of environments, including rich and poor sites (Jackson et

al., 1990; Grime et al., 1991; Fitter, 1994). Our study results are seemingly consistent with this idea, because morphological and physiological plasticity were clearly demonstrated. However, caution should be taken, since our study included only permanent nutrient patches - not pulsed patches. Additional experiments including pulse and permanent patches would be useful in determining conditions that contribute to particular root foraging behaviors.

Demography and topology are two other possible avenues for roots to exploit heterogeneous soil resources that are not explored in this study. Root topology changes with respect to nutrient treatment and species (Fitter et al., 1988). Root demography is a source of plasticity. Roots that are placed in enriched sites may have a shortened lifespan as compared to roots in unenriched soil (Gross et al., 1993, Pregitzer et al., 1993). One hypothesized explanation for increased turnover is that young roots are more efficient at the uptake of nutrients than older roots, and rapid turnover keeps young, efficient roots working in the patch. Conversely, other studies have shown increased lifespan in enriched sites (Aber et al., 1992, Nadelhoffer et al., 1985, Nadelhoffer, 2000). If roots have a longer lifespan they might be just as effective at excluding neighbor roots as a plant that proliferates roots quickly, but die quickly. Root topology and demography are difficult to measure and there are sparse data reporting response of both to soil resource heterogeneity. The importance of demography and topology in heterogeneous environments has yet to be determined.

Overall, it appears that in these four species, both morphological and physiological plasticity impact nitrogen uptake when nutrient patches are relatively stable in time. Furthermore, the degree of either type of plasticity is not related to growth rate.

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Chapter Three: Influences of nutrient heterogeneity and root foraging behavior on competitive outcomes among four coastal plain species

ABSTRACT

A two-year field study examined effects of nutrient heterogeneity on competitive interactions of four coastal plain species. Monoculture, two-, three-, and four- species plots were established under two spatial nutrient arrangements: homogeneous and heterogeneous. Proliferation of roots in nutrient rich patches (foraging precision), sensitivity of monocultures to heterogeneity, and competitive ability of plants were assessed in the fall of each year. In the first year, all four species were relatively precise foragers for nutrients, but one was more precise than others, according to RFRMD (relative fine root mass difference), an index of precision. In the second year, three of four plant species were precise according to RFRMD. In monocultures, we found no aboveground benefit (sensitivity) to precise foraging behavior of roots when nutrients were heterogeneous. However, one species had significantly greater variability of response in the homogeneous nutrient treatment, compared with heterogeneous, a finding in contrast with previous studies. In two-species plots, heterogeneity significantly ($P < 0.05$) impacted biomass of target plants per gram of neighbor biomass, in the first year, but not in the second. Four-species plots (which were harvested after one year) and three-species plots (harvested after two years), showed significant heterogeneity effects according to Goldberg's D_i, an index based on relative yields in mixture minus expected relative yields derived from monocultures. Overall, effects of nutrient heterogeneity on competition in our study were weak. Likely, heterogeneity plays a relatively minor role in competitive interactions at the establishment phase of succession.

Key Words: nutrient heterogeneity, competitive interactions, coastal plain species, neighborhood analysis

INTRODUCTION

During succession, the soil environment is patchy at the scale of individual plants. Debate continues regarding the significance of the patchiness with respect to a variety of issues including species richness, competition and community structure. Pioneering works (Drew et.al., 1973; Drew, 1975; Drew and Saker, 1975) showed that plants change form and function in response to spatially patchy nutrients. Follow up studies have shown that roots display varying degrees of several types of foraging behaviors including morphological plasticity (Fitter, 1985; Eissenstat & Caldwell, 1988; Mou et al., 1995; 1997; Fransen et al., 1998; Einsmann et al., 1999; Robinson et. al., 1999), physiological plasticity (Jackson et al., 1990, Jackson & Caldwell, 1996; vanVuuren et al., 1996; Robinson et al, 1994;) and demographic plasticity (Aber et al, 1985, Nadelhoffer et al, 1985; Gross, et al., 1993; Pregitzer et al, 1993; Nadelhoffer, 2000). These studies highlight the idea that patchiness of the soil environment may shape competitive interactions, and thus community structure. Finally, the overall variability of root response may increase in heterogeneous soil conditions (Casper and Cahill, 1998). It has also been suggested that particular foraging mechanisms are related to plant form, successional status, or productivity of the site. However, at present there are not enough data to support any particular pattern or hypothesis.

Root foraging behavior may be a key to interspecific interactions because belowground competition is strong in most plant communities, especially those that are nutrient limited (eg. Fitter 1985; Vogt et al., 1986). A synthesis of 23 studies, suggests that belowground dynamics are comparatively more important that aboveground (Wilson 1988). The influence of foraging behavior on competition may be neighbor-specific or condition-specific. For example, in Chapter 1, *P. taeda* growing in monoculture, or growing with *E. hieracifolia* proliferated roots in nutrient rich patches. However, when grown with *S. altissima*, *P. taeda* roots did not proliferate (Chapter 1). During a drought, root growth may increase in deeper soil horizons at the expense of growth in shallow horizons where nutrients are more abundant. Thus, several external factors may

significantly affect root foraging behavior, and therefore the potential intensity of root competition between neighbors.

Long-term field studies that include more than one species are needed to determine influences of foraging behavior and nutrient heterogeneity on competitive interactions. Much of the literature on foraging behavior is based on monoculture short-term field work, or pot studies (Casper et al., 2000). Most of these studies have neglected to include competition, or have limited the investigation to a single, species pair. Many of these studies have shown no influence of heterogeneity on mean population responses, although heterogeneity can increase within population size variability (Casper et al., 2000). In a natural setting, plants grow interspersed with a variety of species. In a previous short-term field experiment, we found that precise foragers had increased competitive ability when soil nutrients were distributed in patches instead of uniformly (Chapter 1). However, drought effects were an unintended part of that experiment, and the design was complicated by planting disparate life forms in two-species plots. In this experiment, plants were grown in monoculture, two-species, three-species and four-species combinations and monitored over two growing seasons in a field setting. In two-species plots, similar growth forms were paired.

Our objective was to determine if soil nutrient heterogeneity influences competitive outcomes among four co-occurring plant species. Our first hypothesis is that because plants differ in root foraging ability, heterogeneity of nutrients will influence competitive outcomes. We predict that species which are morphologically precise are also sensitive to nutrient heterogeneity (evidenced by greater aboveground biomass in the heterogeneous nutrient condition). Similarly, these same species should be stronger interspecific competitors where soil nutrients are heterogeneous then where they are homogeneous. Our second hypothesis is that variability of plant size will increase in heterogeneous nutrient conditions compared to homogeneous. Non-uniform resource conditions should increase plant variability since all roots are not exposed to the same level of fertility. Highly fertile microsites which are available to roots that forage for

them, could increase plant growth, resulting in asymmetric competition for aboveground light.

METHODS

Species and study site

We chose four early to mid successional species that co-occur in warm-temperate, coastal plain forests of the southeastern USA: an annual (*Erechtites hieracifolia* (L.) Raf.), a perennial (*Solidago altissima* L.), and two trees (*Pinus taeda* L. and *Liquidambar styraciflua* L.). During the first year of growth after seed germination, *E. hieracifolia* and *S. altissima* grow rapidly to one or more meters in height, while the two trees (*L. styraciflua* and *P. taeda*) produce shorter stems (0.5 m or less). During the second year of growth, trees typically attain heights greater than one meter and the perennial, *S. altissima,* can grow to 1.5 m or more in height.

The experiment was conducted in an old field at the Joseph W. Jones Ecological Research Center at Ichauway, Baker County, GA. Soils are Lakeland Series (sandy, thermic, coated, Typic Quartzipsamments). In May 1998, the field plot was divided into four 15 x 11.5 m blocks to control for trends in soil conditions. After the blocks were disked to a depth of 20 cm and rolled to repack the soil, all vegetation and seeds were killed by fumigation with methyl bromide (98%) and chloropicrin (2%).

Plant preparation and establishment

Tree seeds were obtained from Flint Nursery in Byromville, Georgia. In October and November 1998, seeds of the herbaceous species were collected from a field near the study site.

From February 28 until April 1 seeds were geminated in a greenhouse at Virginia Polytechnic Institute and State University, Blacksburg, VA, USA. When plants had attained sufficient size to survive transplant (4 cm height, 2 leaves), seedlings were planted into plugs (2.5 x 2.5 wide x 6.3 cm deep) in a 50:50 (by volume) mixture of Metro Mix 200 (Scott's Sierra Horticulture Products,

Marysville, OH) and sterilized sand. To inoculate for native microbes, 1.5 g of soil from the Georgia field site was added to each plug. Plants were watered twice daily, ensuring that the growing medium was kept near field capacity. Plants were fertilized April 16th with a liquid 20-20-20 (N-P-K) fertilizer solution (100 ppm). On May 12-15, all seedlings were transported to the Georgia site and planted into experimental plots using 15 cm spacing. Cylindrical plugs of soil (8.5 cm deep by 2.5 cm width) were removed from the plots using a sink pipe to make uniform holes; and plants were placed within the holes. Soil was gently compacted around the plants and any surplus soil from the extraction was discarded outside of the plot.

Experimental design

The experiment included four species, two nutrient treatments (homogeneous and heterogeneous) and 2 levels of competition: intraspecific (monocultures), and interspecific (2-species plots, 3-species plots, and 4-species plots) (Fig 1, Table 1). All plots containing trees were double planted for harvest in 1999 and again in 2000 (Fig 1, Table 1).

In monoculture plots, eight target plants were surrounded by a double border row of plants. In two-species plots, eight plants of one species (target) were surrounded by a matrix of the other species. Two-species plots included: annual x perennial (*E. hieracifolia* x *S. altissima*), and tree x tree (*L. styraciflua* x *P. taeda*). In both comparisons, species were targets in half of the plots and used as matrix species in the other half. Three and four species interspecific competition plots were planted using a Latin square design with a double border row (plant at each location determined randomly) surrounding an inner matrix of target plants (Fig 1, Table 1).

Fertilizer was distributed across plots in two arrangements: homogeneous and heterogeneous. Each provided a mean of 2.1 grams of Osmocote slow release fertilizer (17-9-12 plus minors, Scott's Sierra Horticulture Products, Marysville, OH) per m^2 soil. The nutrient release rate from this quantity of fertilizer is

Figure 3.1: Configurations of garden plots to test nutrient heterogeneity effects on competition. (A) shows an herbaceous monoculture plot. Eight target plants are surrounded by border plants (O) of the same species. (B) shows the design of tree monocultural plots. Notation as in (A). Plots were double planted for harvest in 1999 and 2000. (C) shows a four-species plot that consisted of an inner 4 x 4 area where targets are arranged in a Latin Square Design and surrounding border rows containing a random mixture of the four study species. Two-species plots containing herbaceous species (D) and trees (E), are similar, but tree plots are double planted for separate harvests in 1999 and 2000. Squares at opposite corners of plots represent selected neighborhoods containing target plants 1 & 8 and all nearest neighbors. All plots were treated with uniform nutrient distribution (homogeneous treatment, not shown), or with patches of fertilizer placed such that each plant was 10.8 cm from the closest patch (heterogeneous treatment, squares in C).

A

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B

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O O 1 2 3 4 O O

O O 5 6 7 8 O O

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O O O O O O O O

O O 1 2 3 4 O O

O O 5 6 7 8 O O

O O O O O O O O

O O O O O O O O

C

O O O O O O O O

O O O O O O O O

O O 1 2 3 4 O O

O O 4 3 1 2 O O

O O 3 4 2 1 O O

O O 2 1 4 3 O O

O O O O O O O O

O O O O O O O O

D

O O O O O O O O O O O

O O O O O O O O O O O

O O 1 O 2 O 3 O 4 O O

O O O O O O O O O O O

O O 5 O 6 O 7 O 8 O O

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E

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O O 5 O 6 O 7 O 8 O O

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O O 1 O 2 O 3 O 4 O O

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Table 3.1: Size and content of garden plots used to test nutrient heterogeneity effects on competition.

Competition type	Target plants	Matrix plants	Total plants	Plot Dimensions (cm)	Total Plots
Intraspecific herbs	8	40	48	90 x 120	16
Intraspecific trees	16	64	80	120 x 150	16
Interspecific herbs (2 species)	8	69	77	105 x 175	16
Interspecific trees (2 species)	16	117	133	175 x 190	16
Interspecific (3- species)	9	40	49	105 x 105	8
Interspecific (4 species)	16	48	64	120 x 120	32

comparable to natural N-mineralization rates found in nutrient poor, sandy, coastal plain soils (Burger and Pritchett, 1984; Bell and Binkley, 1989). In homogeneous plots, fertilizer was tilled evenly into the surface 3 cm of soil across the whole plot including a 15 cm buffer outside the plot. In heterogeneous plots the same total fertilizer was mixed into the top 3 cm of selected 5 x 5 cm patches representing ~1 % of surface area. Each plant was 10.8 cm away from the center of one nutrient patch (Fig 1).

Landscape cloth was placed between plots to preclude weed growth. The area within plots was hand weeded biweekly. To ensure adequate water supply, an irrigation system was set up with sprinkler heads placed in the center of each plot. Plots were watered daily from 12-6 am in the absence of natural rainfall. Sprinkler heads were mounted on dowel rods as plants grew in order to project water outward to the entire plot without interception by tall foliage. Evenness of moisture was measured on July 13, 1999 by gravimetric soil analysis.

June 23-26, 1999 anion and cation exchange membranes (each 3.8 x 3.8 cm) were inserted into the top 5 cm of soil in monocultures. In heterogeneous plots, membranes were placed in nutrient rich and nutrient poor soil patches, while in homogeneous plots, membranes were placed in random locations. The adsorption of nutrients by the membrane is a measure of nutrient availability to the plant roots (Abrams and Jarrell, 1982). Membranes were left in the plots for 72 hours, removed and extracted with 25 ml (per membrane) of 2.0 M KCl and then the extract was analyzed by LACHAT QuickChem AE autoanalyzer (Zwellinger Analytics, Inc Milwaukee, WI) for nitrate and ammonium concentrations.

Plant measures and harvest

Plant height of all targets, and eight selected matrix plants were measured June 23, September 9, 1999, August 8, and September 7, 2000. Percent of plot covered by foliage was also estimated for each plot on June 23, 1999.

Individual target plants were harvested on September 9-11, 1999 (all plots) and September 7-8, 2000 (tree monoculture, tree 2-species, and 3-species

plots). In two-species plots, two target plants at opposite ends of each plot (numbered 1 & 8, see Fig 1) were selected for neighborhood analysis. All of the matrix plants (excluding border plants) were also harvested. Oven dry biomass (60 °C) of the entire shoot was determined for each target plant, and dry mass and number of surviving plants were determined for the matrix plants in the two neighborhoods, and for the rest of matrix plants (Fig 1, Table 1). Soil cores (10.2 cm diameter x 20 cm deep) were harvested from randomly located patches in homogeneous plots, and from randomly selected nutrient rich and nutrient poor patches within heterogeneous plots. Cores were rinsed through a 2 mm mesh, and then roots were removed by hand sorting. Roots were dried at 60 °C for 72 hours and then weighed.

Analysis

Statistical Analysis System (SAS) software (SAS version 8.0, SAS Institute Inc., Cary, NC) was used for all statistical analyses. The experiment was arranged as a randomized complete block design. We chose to block based upon differences in light conditions and soil nutrient availability. Significant differences in Potassium were found across blocks, although OM content, pH, and other nutrient levels were similar (Table 2). Data were log-transformed when needed to correct for skewed distributions; however only non-transformed data are plotted in figures.

Precision of root foraging for each species was estimated by root mass in nutrient rich cores versus that in nutrient poor cores in heterogeneous monoculture plots (1999 and 2000) and cores in two-species plots (2000). A two-way ANOVA was used with these data to detect treatment and species (1999) or species/species combination (2000) differences in root biomass. A second analysis of precision was based on RFRMD (modified from Mou et al., 1995). RFRMD was equal to the difference between root core biomass in nutrient rich and nutrient poor patches, divided by the total root core biomass. Confidence interval estimates were used to determine if ratios were different from 0, the value expected for no precision. .

Table 3.2: Mean concentrations (ppm) of soil parameters measured for each of four blocks at study site. P- values are for ANOVA test of block differences (n = 4 samples per block).

Soil parameter	Block 1 (NW corner)	Block 2 (SW)	Block 3 (NE)	Block 4 (SE)	P-value
P	34 +/- 8.25	38 +/- 10.39	47 +/- 9.54	50 +/- 9.17	P > 0.05
K	138 +/- 11.45	188 +/- 14.70	170 +/- 25.38	203 +/- 26.00	p = 0.003
Ca	576 +/- 173.07	612 +/- 124.71	804 +/- 156.92	600 +/- 24.00	P > 0.05
Mg	106 +/- 25.06	107 +/- 14.53	127 +/- 14.80	115 +/- 11.79	P > 0.05
Zn	3.2 +/- 0.85	3.7 +/- 0.66	3.2 +/- 0.49	4.1 +/- 0.71	P > 0.05
Mn	39.5 +/- 4.39	51.8 +/- 0.85	46.6 +/- 9.24	43.2 +/- 2.16	P > 0.05
Organic Matter	1.25 +/- 0.50	1.0 +/- 0.00	1.5 +/- 0.50	1.25 +/- 0.61	P > 0.05
Ph	5.75 +/- 0.39	5.68 +/- 0.37	6.0 +/- 0.32	5.8 +/- 0.22	P > 0.05

Sensitivity analysis was conducted using aboveground biomass in the monocultures. A two-way ANOVA was used to test for nutrient treatment and species effects on biomass.

Nutrient and competitive effects in two-species plots were determined by a ratio of target biomass per gram of competitive neighbor biomass. The ratio was compared in heterogeneous and homogeneous nutrient conditions. Effects of nutrient heterogeneity on interspecific competition were tested using a one-way ANOVA for each nutrient arrangement. The two combinations tested were: *S. altissima* x *E. hieracifolia* and *P. taeda* x *L. styraciflua*. Target plant biomass relative to neighborhood biomass was used as the response variable in this analysis. Data from the two neighborhoods in each plot were averaged, and each plot was considered a single replicate.

To analyze effects of spatial nutrient treatments on the three and four species communities, we calculated Goldberg's competition index ($D_i = RY_{ix}$-RY_{im}), a measure of each plant species' competitive ability within the community. RY_{im} is the relative yield of species *i* in monoculture (calculated by the yield for a species in monoculture divided by the sum of the monocultural yields for all species). RY_{ix}, the relative yield of species *i* in mixture, is calculated similarly with the yield for a species in mixture divided by the sum of yields for all species in the mixture. The D_i values were calculated by subtracting average RY_{im} (by species, averaged across the plots) from the RY_{ix} value for each plot. If D_i is zero, interspecific and intraspecific competition is equivalent for that species. D values were analyzed by a one-way ANOVA with nutrient treatment as the main effect. Comparisons between species were not possible due to limitations of the experimental design.

RESULTS

Soil and plant measures

In 1999, soil moisture averaged 9.1 % and the range was from 6.1-12.1%. In 2000, soil moisture ranged from 6.2-13.6% with a mean of 9.0%. Mean ammonium and nitrate values differed with respect to treatment, but not with

respect to species. Mean ammonium in fertilized patches of heterogeneous plots averaged 25.89 ppm (se=2.27) while nitrate averaged 29.63 ppm (2.33). These values were significantly greater than in homogeneous plots where mean ammonium and nitrate were 1.84 (0.15) and 3.87 ppm (0.39) and in unfertilized areas of heterogeneous areas where values were 2.10ppm (0.15) and 3.72 ppm (0.09) respectively. For both nutrient types, no significant differences occurred between the latter two nutrient treatments ($p > 0.05$).

In the first year, mean plant height in monoculture for both *S. altissima* and *E. hieracifolia* exceeded 1 meter (1.88m (se= 0.24) and 1.12m (0.21) respectively), while mean tree heights for *P. taeda* and *L. styraciflua* (0.33m (0.12) and 0.25 m (0.68)) were significantly less ($p=0.0001$; df = 3,27). In the second year, tree monocultures were harvested and there were no species or treatment differences ($p > 0.6$; df=3,27). Mean height of *L. styraciflua* was 1.12 m (se= 0.29) ; and mean height of *P. taeda* was 1.16 m (0.15). Plant survival was high (99.9%) in the first growing season, and in the second was high (99.8 %) for the first growing season, and in the second was high (99.8%) for all except the three species plots (93%). By August, 1999, canopies in all plots were closed and percent cover was 95-100%. Canopies were surveyed again in August 2000, and percent cover was 100% at all plots.

Precision and sensitivity

All species were precise foragers according to root core biomass data collected from heterogeneous monocultures in the first year. Two-way ANOVA revealed both species ($p =.0003$, df = 3) and treatment effects ($p = 0001$, df = 1; Fig 2). All species had significantly greater biomass in high fertility patches (overall mean of 3.03 g) than in unfertilized areas (1.35 g) within heterogeneous plots (Fig 2). The data from the trees after the second year showed similar results. Both species ($p = .012$. df = 3) and treatment effects were significant ($p = .0003$; df =1) and all species/species combinations responded similarly with greater root biomass in fertilized patches (Fig 3). However, according to RFRMD (relative fine root mass difference) confidence interval estimates, in the first year, only one

Figure 3.2: Dry root biomass from soil cores collected in high and low fertility patches of monoculture plots in 1999. Error bars are standard error based on 32 plots. Abbreviations are as follows: Erhi = *Erechtites hieracifolia*, List = *Liquidambar styraciflua*, Pita = *Pinus taeda*, and Soal = *Solidago altissima*).

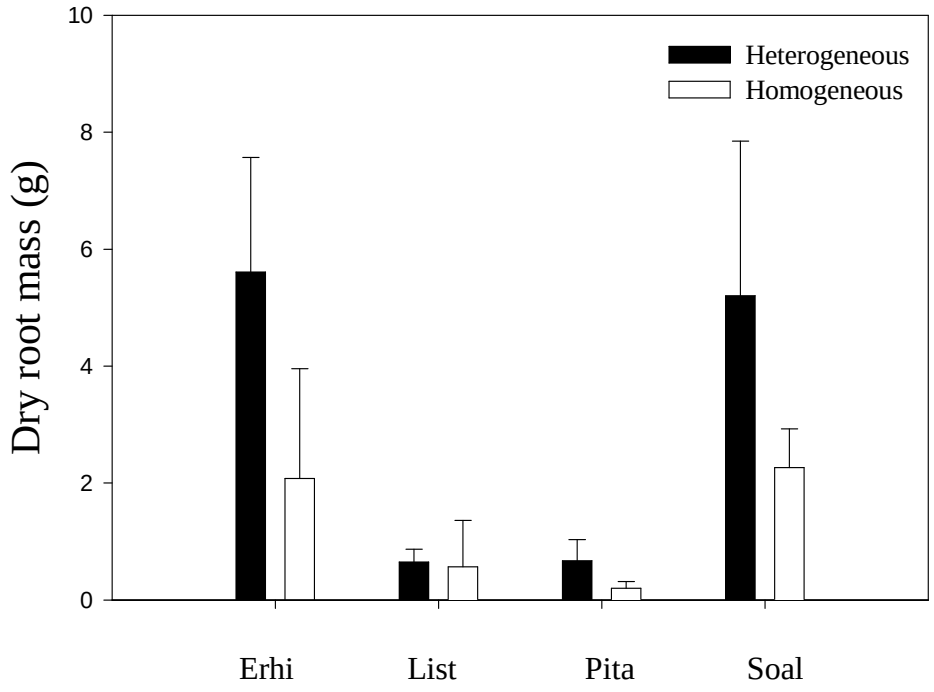
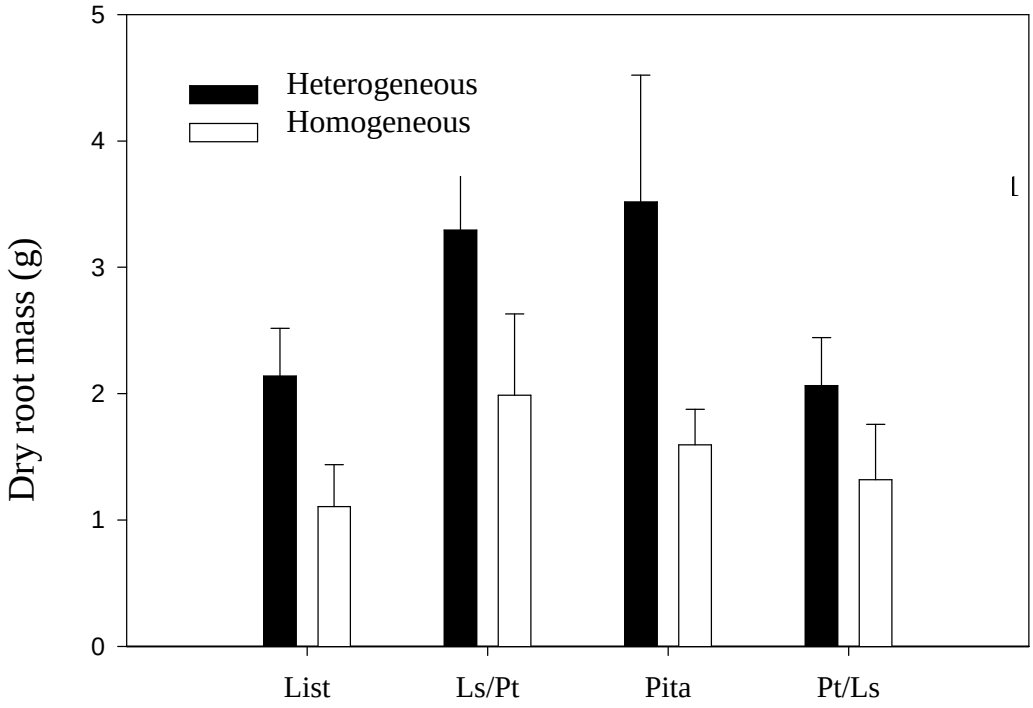


Figure 3.3 Dry root biomass from soil cores collected from high and low fertility patches in monoculture and two-species plots in 2000. Error bars are standard error based on 32 plots. Abbreviations as above for List and Pita. Ls/Pt = *L. styraciflua* target biomass (when grown with *P. taeda*). Pt/Ls = *P. taeda* target iomass (when grown with *L. styraciflua*).



species, *S. altissima* was precise. In contrast, in the second year, three of the four species showed significant precision by confidence interval estimates (Table 3).

According to analysis of monocultures, no species was sensitive to nutrient arrangement. In both 1999 and 2000, significant differences among species were found ($p < 0.05$; $df = 3$), but no significant nutrient treatment effects were detected ($p = 0.47$, (log transformed, for 1999); $p = 0.51$; for 2000).

Interspecific competition

Nutrient arrangement did not influence competitive interactions. In the first year, a two-way ANOVA based on log-transformed values revealed only species effects ($p = 0.803$, $df = 3$, Fig 4). Competitive ability of species pairs was not affected by heterogeneous versus homogeneous nutrient arrays (Fig 4). In the second year, both tree species showed the same trend (greater growth in the heterogeneous condition), but again, without significance ($p = 0.593$, $df = 3$).

In four species plots, nutrient arrangement had a significant impact on competitive outcome. There were significant nutrient effects for *E. hieracifolia* and *P. taeda* ($p < 0.001$ $df = 1$, figure not shown). *Erechtites hieracifolia* was the only species that had a positive D_i value, indicating that it was a better competitor in the mixed species plot than in monoculture, while the three other species had lower yield in the mixed species plot than in monoculture. *Erechtites hieracifolia, S. altissima* showed the trend of greater interspecific competitive performance under homogeneous nutrient conditions, while *P. taeda* and *L. styraciflua* appeared to grow the same, or worse under homogeneous nutrient conditions.

All three species had greater relative yield in the three-species plots compared with monoculture (based on positive D_i values) (Fig 5). Treatment effect was highly significant for *L. styraciflua* ($p < 0.001$ $df= 1$). *Liquidambar styraciflua* clearly had greater competitive success in the homogeneous treatment, while *P. taeda* and *S. altissima* appeared to have nearly the same competitive ability in both nutrient treatments (Fig 5).

Table 3.3: Confidence limits for RFRMD (relative fine root mass difference) in heterogeneous monoculture plots.

Species	Year	Confidence Limits	(CL)	CL not equal to 0 ?
E. hieracifolia	1999	-0.218	1.023	No
L. styraciflua	1999	-0.299	0.916	No
P. taeda	1999	-0.008	0.954	No
S. altissima	1999	0.053	0.228	Yes
L. styraciflua	2000	0.051	0.697	Yes
L. styraciflua (in competition with P. taeda)	2000	0.065	0.478	Yes
P. taeda	2000	0.137	0.598	Yes
P. taeda (in competition with L. styraciflua)	2000	-0.025	0.352	No

Figure 3.4 Neighborhood analysis of two-species competition plots. Notation on the abscissa indicates species pairing with target species listed first. Ordinate shows target biomass divided by mean neighbor plant biomass. Abbreviations as in Figure 2. Error bars are standard errors based on n = 64 neighborhoods.

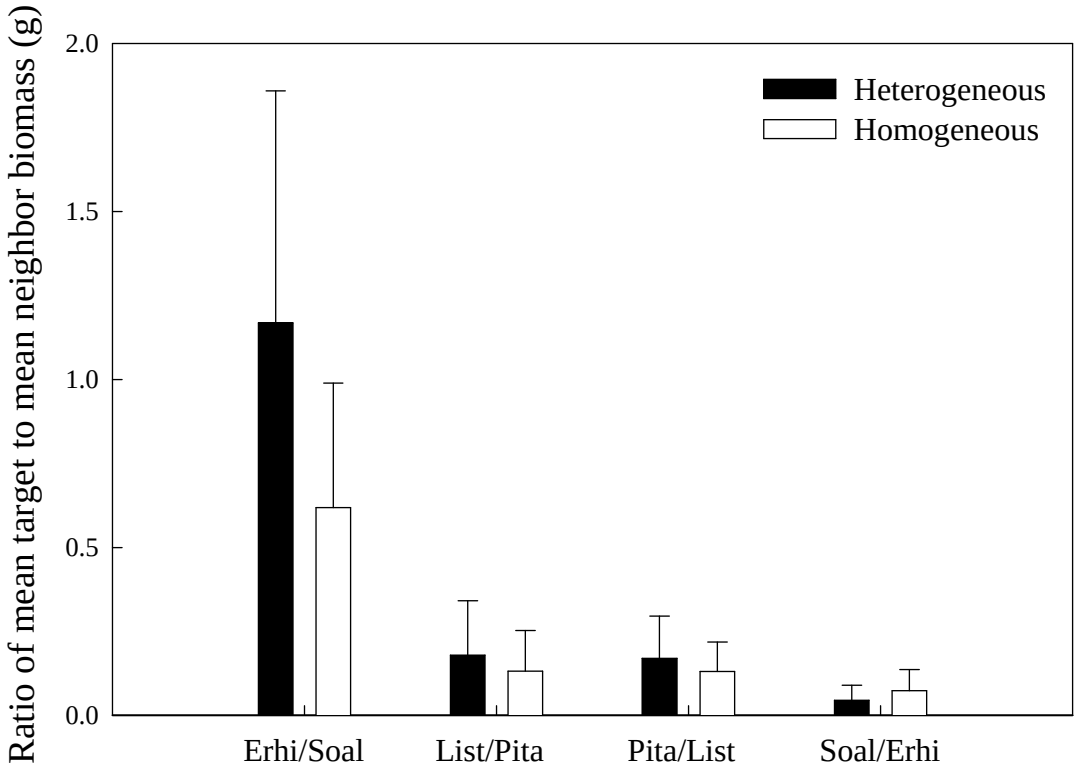


Figure 3.5 Goldberg's D_i index (relative yield of plants in mixture minus relative yield in monoculture) for three-species plots. Species abbreviations as in Figure 2. Error bars are standard errors based on n = 12 plots.

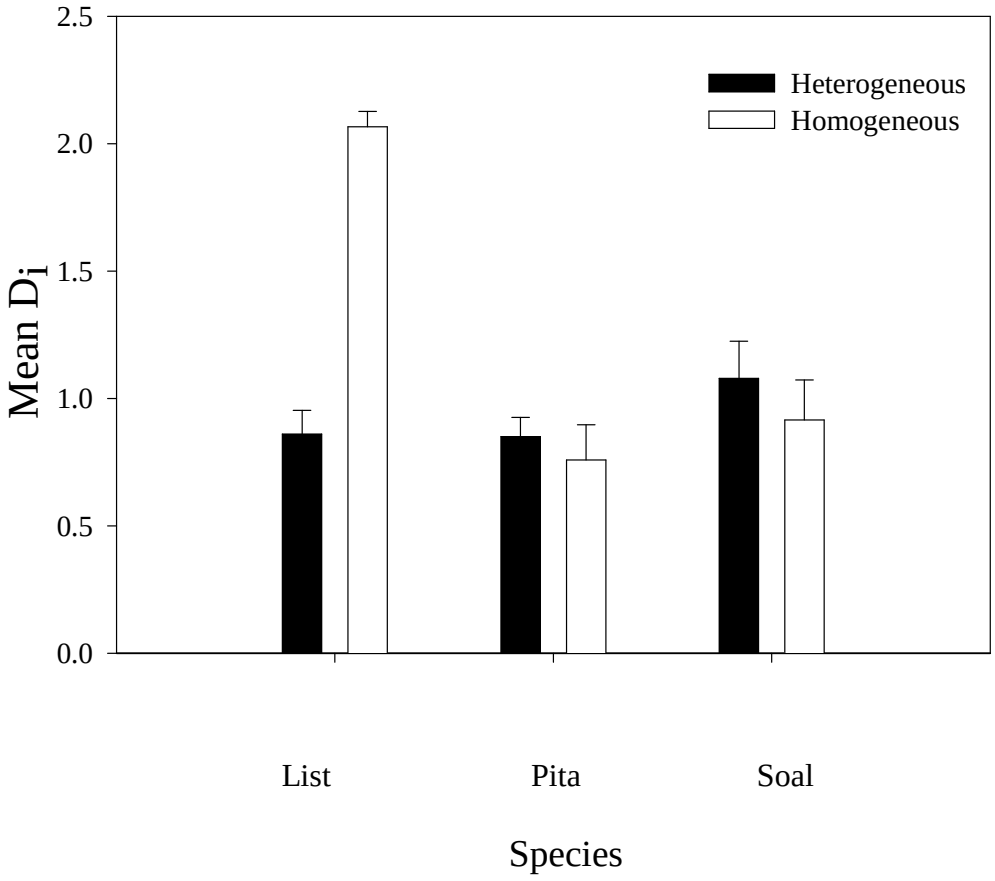
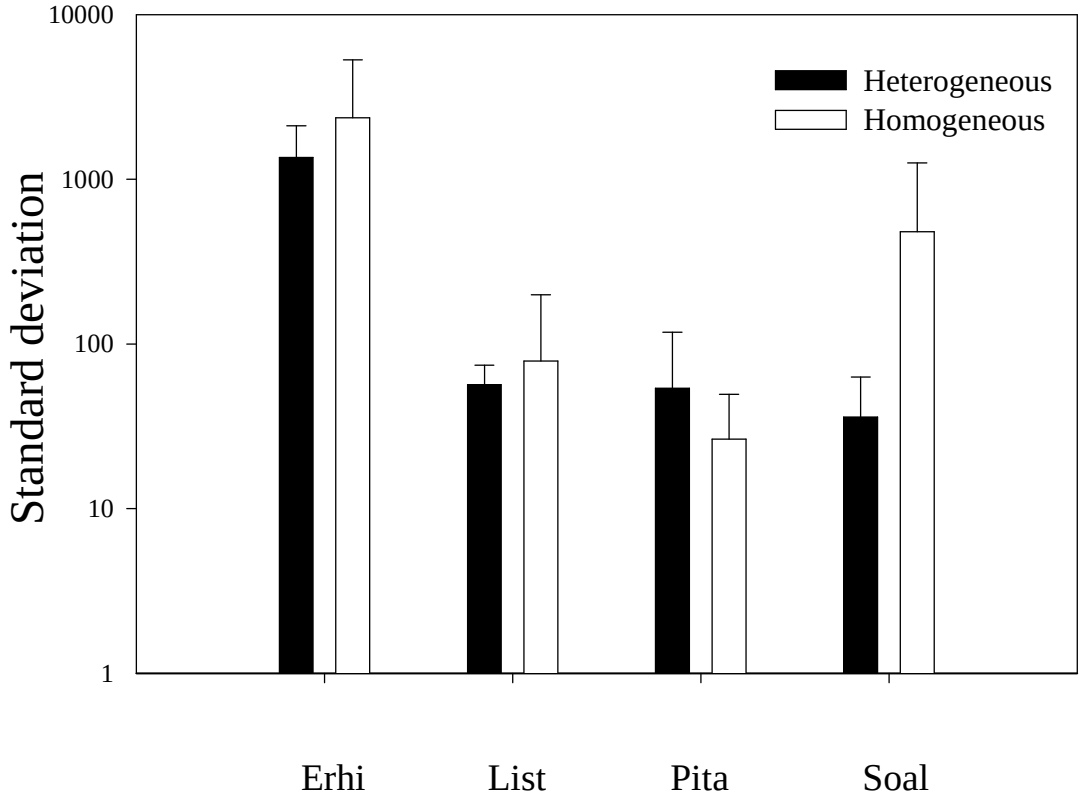


Figure 3.6 Standard deviation of aboveground plant biomass for target plants in plots with heterogeneous or homogeneous nutrient arrangement; n= 4, error bar is standard error. Species abbreviations as in Figure 2



Variance and how it relates to heterogeneity and competition

In all four species, standard deviation of biomass within monoculture plots (based on the eight target plants) was greatest for the two species with greatest mean biomass (Fig 6). Acording to an ANOVA, species effects were significant ($p = 0.0001$; $df = 1,3$; Fig 6). However, treatment effects were not significant ($p = 0.76$). Trends showed that *Solidago altissima* had greater variability in the homogeneous treatment, while the other species showed similar responses, independent of treatment.

DISCUSSION

Our prediction that species that are morphologically precise are also sensitive to nutrient heterogeneity was not supported. In 1999, three species were precise, yet none were sensitive. In 2000, both trees were precise, but again, neither was sensitive. It appears that in monocultures, precision does not produce a corresponding gain in aboveground biomass. Why then, do plants show morphological plasticity?

The answer lies in the mixed species plots. Our first hypothesis was that heterogeneity of nutrients influences competitive outcomes. Limited support for the hypothesis comes from three- and four-species plots. Four-species plots (harvested in 1999) showed that two of the four study species improved their relative yield in heterogeneous nutrient conditions, while three-species plots (harvested in 2000) showed a treatment effects for just one species. In both three- and four-species plots, D_i values were positive, indicating that plant species relative yield was higher in mixture than in monoculture. Release from intraspecific competition pressure allowed all species enhanced yield. However, two- species plots (1999 and 2000) showed that heterogeneity of nutrients did not enhance growth of any of the study species. Our data suggest that improved yields in mixture are primarily the result of interspecific competition, and that heterogeneity may provide short-term (establishment phase) benefits, but not long term benefits.

Further, since interspecific competition is more common than intraspecific in nature, nutrient heterogeneity probably has a measureable effect in at least

some natural communities (as suggested by vanVuuren et al. 1996). However, our findings also agree with other studies that implicate factors other than heterogeneity as being more important in competitive interactions (Larigauderie and Richards, 1994; Cahill and Casper, 1998; Ryel and Caldwell, 1998). We say this because the responses to nutrient arrangement in our study were relatively small and in the case of trees, only lasted one growing season. It is probable that in the establishment phase of succession, root foraging responses to nutrient heterogeneity do affect competitive interactions. However, the effect is small in magnitude and temporary, and likely shifts in response to changing biotic and abiotic conditions.

Our second hypothesis, that heterogeneous conditions increase variability of response was not supported. Our data show that variability of response is not related to heterogeneity, but rather is a function of species. Contrary to our hypothesis (and to findings by Casper and Cahill, 1998), the trend for one species was for variability to increase in the homogeneous treatment. Variance was a function of species and there was a tendency for larger plants (herbaceous species) to have higher variability.

An unexpected finding was that water can influence competitive outcomes in conjunction with foraging. In two-species plots, when water is limiting, some plants (*E. hieracifolia*, *P .taeda*) do not show precise foraging for nutrients (Chapter 1), however when water is not limiting the roots do appear to forage precisely for nutrients. In addition, the water sensitive species (*E. hieracifolia*) was able to increase biomass relative to drought tolerant (*S. altissima*) under heterogeneous, well-watered conditions. In contrast, the opposite pattern, (*S. altissima* increasing biomass in heterogeneous conditions, and *E. hieracifolia* gaining biomass in the homogeneous treatment) existed in drought conditions (Chapter 1). This apparent reversal in foraging behavior emphasizes the importance that resources other than nutrients (such as water) can have on competition in mixed species plots.

In general, our data support the idea that nutrient heterogeneity has the potential to effect competitive interactions. However, this experiment combined

with our findings in previous work and other studies (Mou et al, 1997; Casper and Cahill, 1998; Hodge et al. 1998; Fransen, DeKroon and Berendse, 1998; Ryel and Caldwell, 1998) suggest that outside factors (water availability, light) may be more important than nutrient heterogeneity in shaping competitive interactions.

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Chapter 4: SUMMARY OF FINDINGS

The overall goal of my study was to determine the effects of nutrient heterogeneity on plant root response, and on intraspecific and interspecific competitive outcomes. Soil resource heterogeneity clearly influenced root foraging behavior. Precision differed among species according to competition level (monoculture or mixed species plots) and abiotic conditions, however, almost all species (5 of 6) displayed precision in at least one of the experiments (Chapters 1, 2 and 3). Of the four species grown in both field experiments (Chapters 1 and 3), root precision in monoculture plots was consistently detected for three species. The exception, *E. hieracifolia*, had roots that proliferated in patches only when water was abundant (Chapter 3), not under water-limited conditions (Chapter 1). When the same species were grown in multi-species plots, precision changed. I found that in these species, precision of roots is a generalized response; and suspect that all plants have the ability to proliferate roots in nutrient rich patches. However the ability varies and is dependent upon both biotic (e.g., competition type, neighbor species) and abiotic factors (e.g., water availability).

Our data suggest that plants also respond to heterogeneity with physiological plasticity (uptake, Chapter 2). Our ^{15}N data show that increased uptake occurs, particularly in the low nutrient half of heterogeneous pots. In particular, physiological plasticity helps plants equalize uptake of N relative to amount of roots in the patch. To illustrate, the same total amount of ^{15}N was absorbed by the plant regardless of fertility arrangement (high fertility half – dense roots or low fertility half – sparse roots). By increasing uptake, plants that are in a nutrient poor environment take advantage of nutrient pulses with less carbon investment. This mechanism may be less important to plants that are fast growing, or in plants that are in a nutrient rich environment. However, based on studies quantifying root turnover and structure, I speculate that

demography and topology may also be key factors to study when analyzing root responses to heterogeneity.

Sensitivity, total plant biomass in monoculture, was not affected by soil heterogeneity (Chapters 1, 2 and 3). Sensitivity was not strongly related to either precision or profusion. Apparently, aboveground biomass in monoculture is relatively unaffected by heterogeneity - even in species with precisely foraging roots. Thus, when same species neighbors are present, all plants use the same strategy (precision) to gain nutrients, and there is no overall gain in fitness. Contrast that situation with a field setting, where many different plants (presumably with capacity to use different foraging strategies) are competing, and it is easy to see that sensitivity could play a role. My findings on monoculture response contrast sharply with a previous study from our lab (Einsmann et al., 1999) and are similar to a number of others (Casper et al. 2000). However, caution should be exercised in interpreting and generalizing plant monoculture responses with overall plant fitness or competitive ability. Studies examining root and aboveground behavior in a mixed community would provide the best data to answer questions about sensitivity.

Although neither profusion nor precision was strongly related to competitive ability (measured as aboveground biomass, Chapter 1), there is some evidence that root precision does provide an advantage in interspecific competition plots (Chapters 1 and 3). In Chapter 1, two of the 2-species combinations showed that precise roots conferred an increase in aboveground biomass in heterogeneous nutrient conditions. In Chapter 3, there was a tradeoff with *E. hieracifolia* having greater biomass in the heterogeneous treatment, and *S. altissima* having greater biomass in the homogeneous treatment. The trees appeared to gain aboveground biomass under heterogeneous conditions, however, after the second year (when competition was more intense due to increased size) there was no significance. So, the aboveground advantage afforded by precision may

be neighbor specific (two species, *S. altissima* and *E. hieracifolia* increased biomass under homogeneous or heterogeneous condition depending upon neighbor species), and other biotic (e.g., physiological and demographic plasticity) and abiotic factors (e.g., sunlight, water availability) could be as important, or more important in determining competitive ability.

In plots with 3, 4 or 6-species combinations, some nutrient effects were detected. In 1998, species effects overwhelmed nutrient effects in 6-species plots (Chapter 1). In 1999-2000, 3 and 4-species plots were established with higher replication in order to increase the power of the experiment. In 3-species plots, *L. styraciflua* had increased yield in the homogeneous treatment compared to heterogeneous treatment (grown with neighbors *S. altissima* and *P. taeda* – all precise foragers). In 4-species plots, *E. hieracifolia* increased yield in the homogeneous treatment compared with heterogeneous (all neighbors – *L. styraciflua*, *S. altissima*, and *P. taeda* were precise foragers). The significant nutrient effects in 3 and 4 species plots were a function of a large response of one species relative to the others (2000- *L. styraciflua*, 1999-*S. altissima*). So, shifts in competitive interactions were not observed for most species (although we did see this for one species each year), and differences that were detected showed increased relative yield in homogeneous conditions for both of those species. Therefore, it appears that neither root precision nor heterogeneity of nutrients causes radical changes in competitive ability of species in a mixed community plot.

Several factors outside of the experimental design may confound the experiment. First, size of plants may be factor in interspecific competition experiments. If plant sizes of different species are not matched initially, then asymmetric growth responses may occur that do not reflect nutrient conditions. Further, growth rates of plants may make comparing dissimilar lifeforms complicated. Harvesting plants at about the same size helps eliminate some of the problem, but harvesting at the

same lifestage might be more appropriate (but not feasible). Second, ontogenetic effects were not within the bounds of this study, and could clearly play a role in species response to nutrient heterogeneity. As a plant develops, it may change its foraging strategy over time. Third, abiotic factors may change root foraging behavior. Chapters 1 and 3 provide limited evidence of this phenomenon as exemplified by water. Plant roots can respond differently depending on water availability (one species of four). Other studies have found that abiotic factors may play a more crucial role in competitive outcomes than proliferation does. Therefore, dynamics of patches may partially account for expression of morphological versus physiological plasticity. Specifically, mechanisms of root plasticity may be related to both mobility and longevity of patches.

Overall, there are no clear trends in root foraging response to heterogeneity. 1) Most plants appear able to respond to nutrient heterogeneity with proliferation (Chapters 1, 2 and 3). 2) In addition, some plants may have the ability to increase uptake in a patchy nutrient environment (Chapter 2). 3) However, a consistent advantage a plant gains by root foraging behavior has not been demonstrated in the field (Chapters 1 and 3). When plants are grown in monoculture or interspecific competition plots, no increase in aboveground biomass results from precise foraging in heterogeneous conditions. Alternate methods of assessing plant fitness could be explored (reproductive success, root total biomass, nitrogen content) and might clarify the role of root foraging and nutrient heterogeneity in a multi-species setting. 4) Root foraging behavior varies from species to species (Chapters 1 and 3), within a species (depending on neighbors; Chapters 1 and 3), and depending upon water availability (Chapters 1 and 3). 5) Root foraging behavior may change within a species over time, depending upon soil conditions, or in response to number of neighbor plants within a plot. These factors were not a part of this study and should be given consideration in future competition experiments. 6) Furthermore, studies that incorporate

measures of root turnover and topology along with precision and uptake would help in establishing a clearer picture of root foraging responses to nutrient heterogeneity in intra- and interspecific competition.

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Bliss, K.M, Jones, R.H., Mitchell, R.J., Mou, P. Root foraging characteristics: morphological versus physiological plasticity. (in preparation)

Bliss, K.M., Jones, R.H., Mitchell, R.J., Mou, P. Competitive outcomes of early successional species exposed to nutrient heterogeneity. (in preparation)

Cain, R.O., Bliss, K.M., and Jones, R.H. Demographic plasticity in root systems exposed to soil nutrient heterogeneity.

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Virginia Academy of Science 2000 Radford, VA
Poster: Effects of nutrient heterogeneity on plant response and competitive outcomes in early successional species.

Ecological Society of America 1999, Spokane, WA
Poster: Root precision in greenhouse and field experiments indicates that morphological plasticity may confer a competitive advantage.

Graduate School Science Fair 1999, Blacksburg, VA
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1999-2000	Virginia Tech, Graduate research and development grant ($500)
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Botany Seminar Committee

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Biology Graduate Student Association, member at large

Responsible for maintaining the department photo board, organizing and helping out with graduate student events.

Graduate committee for faculty appointment

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