Factors affecting root system response to nutrient heterogeneity in forested wetland ecosystems

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Abstract

Soil nutrients are often heterogeneously distributed in space and time at scales relevant to individual plants, and plants can respond by selectively proliferating their roots within nutrient-rich patches. However, many environmental factors may increase or decrease the degree of root proliferation by plants. I explored how soil fertility, nitrogen (N) or phosphorus (P) limitation, and soil oxygen availability affected root system response to nutrient heterogeneity in forested wetland ecosystems of southeastern United States. Fine root biomass was not correlated with soil nutrient availability within wetland ecosystems, but was related to ecosystem-scale fertility. Root systems generally did not respond to P-rich patches in both floodplain (nutrient-rich) and depressional swamps (nutrient-poor) swamps, but results were inconclusive because the growth medium (sand) potentially hindered root growth. In floodplain forests, roots proliferated into N-rich patches but not P-rich patches, even though litterfall N:P ratios were > 15, which suggested that these ecosystems were P-limited. The combination of nutrient and oxygen heterogeneity affected root proliferation and biomass growth of three common floodplain forest species (*Liquidambar styraciflua*, *Fraxinus pennsylvanica*, and *Nyssa aquatica*) in a potted study, which was related to species' flood tolerance. My results suggest that the environmental context of plants can affect roots system response to nutrient heterogeneity in forested wetland ecosystems and highlights the need for field studies that investigate this phenomenon. Learning how environmental conditions affect plant response to nutrient heterogeneity at a fine-scale will provide better predictions of nutrient cycling, plant competition and succession, and forest productivity, which are important factors that determine carbon sequestration and timber production.

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Chapter 1. Introduction

Soil nutrients are spatially and temporally heterogeneous. Nutrient heterogeneity may be caused by spatial differences in abiotic factors, such as parent material, microclimate, microtopography, soil physical properties, and biotic factors, such as activities of soil organisms or plants (Stark 1994). Ecologists did not explicitly study nutrient heterogeneity until the application of geostatistics to soils began to elucidate spatial patterns of nutrients in ecosystems (e.g. Rossi et al. 1992, Robertson and Gross 1994). It became apparent that nutrients were often variable within the root zone of individual plants (e.g. Jackson and Caldwell 1993a, 1993b, Lechowicz and Bell 1991, Ehrenfeld et al. 1997).

Plants can respond to nutrient-rich patches within their own rooting zone by proliferating roots within those patches. This phenomenon was first formally studied by Drew and co-workers in the 1970's who showed that barley plants were able to increase the number of lateral roots in a nitrate, ammonium, and phosphate patch, but not in a potassium patch (Drew et al. 1973, Drew and Saker 1975, 1978, Drew 1975). Since that time, there has been extensive research on root proliferation in many plant species (see Robinson 1994 for a review). Root proliferation has been shown to vary by species, type of nutrient, amount of nutrient in a patch, spatial distribution of patches, and distance to a patch (Robinson 1994). Empirical studies of the proliferation response have been conducted at the individual (e.g. Robinson 1994), population (Casper and Cahill 1996, 1998, Day et al. 2003a, 2003b), and community level (Bliss et al. 2002).

Root proliferation presumably increases nutrient capture, especially when plants compete for nutrients with other plants (Robinson 2001, Hodge et al. 1999). This behavior may allow plants to efficiently compensate for nutrients that are spatially heterogeneous (Robinson 1994, Hodge 2004). In fact, some studies have shown that certain plants have higher growth when nutrients are heterogeneous than when the same amount of nutrients are homogeneous (Birch and Hutchings 1994, Wijesinghe and Hutchings 1997, Einsmann et al. 1999, Day et al. 2003a, Blair and Perfecto 2004). Also, mathematical models have suggested that nutrient acquisition per gram of root may be more efficient under heterogeneous conditions compared to homogeneous conditions (Jackson and Caldwell 1996).

The benefits of root proliferation are clear, but there may be cases where such behavior is not beneficial. For example, root proliferation into nutrient-rich patches may increase a plant's susceptibility to drought, herbivory, or soil anoxia. Certain environmental factors may also exert strong selection pressure on plants and decrease their ability to proliferate. For example, plants that are adapted to nutrient-poor ecosystems have slow overall growth and root turnover rates (Chapin 1980) that may reduce their ability to respond to nutrient-rich patches compared to plants that are adapted to nutrient-rich ecosystems. Empirical studies have generally supported this prediction (Crick and Grime 1987, Hutchings and de Kroon 1994, Fransen et al 1998, 1999).

In forested wetlands of southeastern United States, hydrology can influence nutrient and oxygen availability at local and ecosystem scales, which in turn may affect root proliferation. In the following chapters, I examine how these wetland soil conditions influence root system response to nutrient heterogeneity. In Chapter 2, I quantified nitrogen and phosphorus heterogeneity and determined whether root biomass was correlated with nutrient availability at local and ecosystem scales. In Chapter 3, I explored how root systems respond to phosphorus heterogeneity in nutrient-rich and nutrient-poor wetlands by manipulating the supply of phosphorus in microsites. In Chapter 4, I examined whether nitrogen or phosphorus limitation affected root proliferation patterns. In Chapter 5, I investigated how the combination of oxygen and nutrient heterogeneity affected root proliferation and biomass growth in three species that co-occur in floodplain forests, but differ in flood tolerance. I found that environmental context, particularly flooding, was important in determining the degree of root system response to nutrient heterogeneity in these wetland ecosystems.

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Chapter 2. Correlations between soil nutrient availability and fine root biomass at two spatial scales in forested wetlands with contrasting hydrological regimes

Abstract

I investigated how two different spatial scales (ecosystem and local) influenced the relationship between soil nutrients and fine root biomass in forested wetlands of southeastern United States. Soil nutrients (NO_3-N, NH_4-N, and PO_4-P) and fine root biomass were measured at three replicate sites in nutrient-rich floodplain swamps, nutrient-poor depressional swamps, and intermediate river swamp sloughs. At one replicate of each wetland type, a dense network of sampling points was used to measure variability (variance and coefficient of variation) and spatial dependence of soil nutrients and fine roots. Fine root biomass was lower in floodplain swamps than either river swamp sloughs or depressional swamps. Multiple linear regression and spearman rank correlations indicated a negative relationship between soil nutrients and fine root biomass at the whole ecosystem scale. Within ecosystem variability of NO_3-N and NH_4-N was greatest in the floodplain swamp, but nutrients were not spatially patchy at the local scale in any of the sampled sites. Within ecosystem correlates between soil nutrients and fine root biomass were generally weak. Soil nutrient availability may control fine root biomass at the ecosystem scale in these forested wetland ecosystems, but it is unclear if the same is true at finer spatial scales.

Introduction

Belowground net primary production (NPP), which may be as much as 50% or more of total NPP (Keyes and Grier 1981, Vogt et al. 1986, Megonigal and Day 1988, Powell and Day 1991, Megonigal et al. 1997), can show rather large variation from one ecosystem to the next. It is unclear if this variation is driven by factors that operate at large, whole ecosystem scales, or alternatively, by the cumulative influence of factors that operate at fine spatial scales within ecosystems. Studies that sample at multiple spatial scales may allow the relative importance of factors structured at broad versus fine spatial scales.

At the whole ecosystem scale, it is well known that the proportion of total plant biomass that occurs belowground is strongly influenced by availability of mineral nutrients. In fertile ecosystems, trees apportion relatively little of their total carbon resources to root production, but the opposite may occur within infertile ecosystems, because more roots are needed to facilitate adequate nutrient uptake (Chapin 1980). Empirical studies have shown a negative relationship between fine root biomass and ecosystem fertility (Keyes and Grier 1981, Aber et al. 1985, Vogt et al. 1987), and fertilization of nutrient-poor forests may reduce ecosystem root biomass (Gower and Vitousek 1989).

Within ecosystems, the fine-scale spatial and temporal distributions of nutrients also influence fine root biomass. Soil nutrients are often substantially variable at scales less than a meter (Jackson and Caldwell 1993a, 1993b, Bell et al. 1993, Ehrenfeld et al. 1997). In response to this variability, many plants selectively increase root biomass within nutrient-rich patches to forage efficiently for nutrients (Drew 1975, Crick and Grime 1987, Campbell and Grime 1989, Robinson 1994, Einsmann et al. 1999). However, most studies linking nutrient patchiness and fine roots have been conducted in greenhouse or garden plot conditions. Few such studies have examined natural forest ecosystems (but see Mordelet et al. 1996).

Soil nutrients have not only direct effects on root response, but also indirect effects by influencing the distribution of plant species and the evolution of root responses within species. Furthermore, nutrient levels at broad scales may influence evolutionary

responses of plants to fine-scale nutrient patterns. For example, fast growing species from nutrient-rich ecosystems have been predicted to show high levels of morphological plasticity (i.e. root proliferation into nutrient-rich patches) to compensate for the high level of nutrient demand of the individual plant and its competitors (Grime 1994). On the other hand, slow-growing species common to nutrient-poor ecosystems are thought to have long-lived root systems that respond to fine-grained nutrient patches through physiological plasticity (i.e. increase in nutrient uptake per unit root length) (Grime 1994, Hutchings and de Kroon 1994). Empirical studies have supported these predictions (Crick and Grime 1987, Hutchings and de Kroon 1994, Fransen et al. 1998, 1999). However, recent evidence suggests that differences in root foraging between fast and slow growing species may be due simply to differences in relative growth rates among plant species rather than to evolutionary specialization (Aanderud et al. 2003).

In forested wetlands of southeastern United States there are distinctly different hydrologic patterns, which influence ecosystem fertility. Non-alluvial wetlands receive most of their external nutrient inputs from precipitation alone and are nutrient poor relative to other forested wetlands. In contrast, alluvial wetlands periodically receive nutrient subsidies from river-flooding and are nutrient rich compared to non-alluvial wetlands. Therefore, these wetlands are good systems to test hypotheses about how fine roots respond to fertility at the ecosystem and local scales. Although the effects of ecosystem-scale fertility on aboveground NPP in wetlands are well known (Brinson et al. 1981, Brown 1981, Mitsch and Rust 1984, Megonigal et al. 1997), effects belowground are not.

In this study, I investigated the relationship between soil nutrients and fine root biomass at two spatial scales (local and ecosystem) in forested wetland ecosystems. Three different wetland ecosystem types (depressional swamps, river swamp sloughs, and floodplain swamps) that vary in their hydrologic regimes were compared. Depressional swamps are non-alluvial systems, whereas river swamp sloughs and floodplain swamps are alluvial. However, the river swamp sloughs used in my study backfill during overbank flow and potentially receive less nutrient laden sediment than floodplain swamps. Here, I consider wetlands types to represent ecosystem scale, and sampling points within sites to represent local scales. I hypothesized that:

1) Ecosystem fertility would be greater in alluvial wetlands compared to non-alluvial wetlands, and fine root biomass should be inversely related to ecosystem fertility. This led to prediction that nutrient availability should follow the trend: floodplain swamp > river swamp slough > depressional swamp, and fine root biomass should follow the opposite trend: depressional swamp > river swamp slough > floodplain swamp.

2) Soil nutrient availability would be spatially patchy in alluvial wetlands due to the influence of present and past nutrient subsidies from river flooding that were not evenly distributed across the wetland. Therefore, the rank order of nutrient heterogeneity for both nutrients and fine roots should follow the rank order of subsidy, i.e.: floodplain swamp > river swamp slough > depressional swamp.

3) Fine root biomass and soil nutrient availability would be positively correlated at the local scale and the strength of the correlation will be directly related to ecosystem fertility. Therefore, the strength of root-nutrient correlations will show the following pattern: floodplain swamp > river swamp slough > depressional swamp.

Materials and methods

Study sites

I identified three geographically distinct depressional swamps (D) and three river swamp sloughs (S) within Ichauway ecological reserve, which is managed by the Joseph W. Jones Ecological Research Center, Baker County, Georgia, USA. Three floodplain swamps (F) were established in the Chickasawatchee Wildlife Management Area, Baker and Calhoun Counties, Georgia, USA. Wetlands at Ichauway have experienced almost no human disturbance since the 1930s (Watt and Golladay 1999). In contrast, most large baldcypress (*Taxodium distichum* (L.) Rich.) in floodplain swamps were removed during the first half of the 20th century.

Basal area of the nine sites measured during my study ranged from 37.6 to 126.7 m^2/ha (Table 2.1). *Taxodium ascendens* Brong. and *Nyssa biflora* Walt. were dominant at depressional swamps. River swamp sloughs were dominated by *T. distichum* at all

sites, *Nyssa aquatica* L. at S2, and *Planera aquatica* J. F. Gmel. at S1 and S3. *N. aquatica* was dominant at floodplain swamps with *Fraxinus pennsylvanica* Marsh., *T. distichum*, and, to a lesser extent, *Quercus laurifolia* Michx. as codominants.

Mean annual temperature is 19ºC, and annual precipitation during the study year was 87 cm, which was 34% below normal (National Climatic Data Center, Asheville, North Carolina). Hydroperiods of forested wetlands in this region generally begin in early spring (March) and extend to early to mid summer (June-July). However, two river swamp sloughs (S1 and S2) were not flooded during the study year and the other sites generally had no standing water by early June. Soils of floodplain swamps and river swamp sloughs are classified as Typic Fluvaquents of the Muckalle series. Soils of the depressional swamps consist of an organic-rich surface horizon and are classified as Histic Humaquepts.

Study design

I randomly selected a 20 × 20 m plot (0.04 ha) within each site in July 2001. Each plot was divided into 2 × 2 m grid cells consisting of 121 grid intersections. At two sites per wetland type, twenty grid intersections were chosen to use as sampling points. At one representative site (D1, S1, and F1) per wetland type, I sampled 80 systematically located points to estimate spatial patterns in nutrients and fine roots. The 80 points included 41 points arrayed in a pattern developed by Halvorson 1994 (Figure 2.1). In addition, three points were located at 0.5 m intervals around 13 randomly selected points of the 41 for a total of 39 additional points. I randomly oriented these groups of three points in a north, south, east, or west direction.

Field measurements

I estimated soil N (NH_4-N and NO_3-N) and P (PO_4-P) availability at each sampling point within plots using ion exchange membranes (IEMs, Ionics Inc., Watertown, Massachusetts). Abrams (1992) showed that P extracted form membranes was strongly correlated with soil P availability. These nutrients were selected *a priori* because they generally limit productivity in forested wetlands within the southeastern United States (Lockaby and Conner 1999). Soil NH_4-N availability was sampled with cation exchange membranes (type CR67-HMR) and NO_3-N and PO_4-P availability were

sampled with anion exchange membranes (type AR204-SZRA for NO_3 and type 204-UZR-456 for PO_4). I cut membranes into 2 × 4.5 cm sections and charged them with 0.5 M $NaHCO_3$. One membrane of each type was inserted into the top 10 cm of surface horizon at each sampling point on 14-16 August 2001 and removed 48 h after installation. NO_3-N and NH_4-N membranes were extracted with 2.0 M KCl and PO_4-P membranes with 0.5 M HCl. Extracts were analyzed for NO_3-N, NH_4-N, or PO_4-P using an autoanalyzer (Lachat Quickchem AE, Lachat Instruments, Milwaukee, Wisconsin).

On 16 to 22 August 2001, I collected 7.62-cm diameter × 30-cm deep soil cores at each sample point within a site to measure root biomass. Roots were removed from the soil by washing the samples with a hydropneumatic root elutriator (Gillison's Variety Fabrication, Benzonia, Michigan) over a 1-mm sieve. Live, fine roots (<2 mm diameter) were removed from each sample and dried at 70ºC to a constant weight.

Statistical Analyses

Hypothesis 1: Ecosystem fertility and fine root biomass

I used MANOVA to test for differences in soil fertility among wetland types with NO_3-N, NH_4-N, and PO_4-P as dependent variables using the GLM procedure of SAS version 8 (SAS institute, Cary, North Carolina). I performed univariate ANOVAs to determine which variables were driving significant results. Alpha for each univariate test was adjusted using a sequential Bonferroni technique to avoid inflation of the Type I error rate. A separate, univariate ANOVA was used to test whether fine root biomass differed between wetland types. Nutrient (NO_3-N, NH_4-N, and PO_4-P) and fine root biomass values were log transformed to meet assumptions of normality. All data in tables and figures are back transformed. At sites D1, S1, and F1 the 80 sampling points were not independent because the spatial arrangement of the sampling points was designed to determine autocorrelation. Therefore, I randomly selected 20 from the 80 points to calculate site means with the condition that each point had to be at least 2 m from an adjacent sampling point. When I repeated this procedure with different random samples of 20 points, results did not change substantially.

I used two techniques to determine if soil fertility was related to fine root biomass at the ecosystem level. First, I used multiple regression analysis with NO_3-N, NH_4-N,

and PO_4-P as independent variables and fine roots as the dependent variable. I selected the regression model with the lowest mean square error as the "best" model using stepwise, forward, and backward regression techniques. Second, I ran Spearman rank correlations between nutrients and fine roots by combining the different types of nutrients into a nutrient index (*en sensu* Jackson 1993b). The nutrient index was created by ranking site means of each nutrient from 1 to 9 and then by summing the ranks for each site. This technique for measuring of relative fertility among sites can reveal relationships not detected by multiple regression analysis, which may suffer from complicated non-linear correlations among nutrients that violate assumptions of independence.

Hypothesis 2: Nutrient heterogeneity

For D1, S1, and F1, I assessed overall, non-spatially explicit variability (i.e., the "global" variability of Lister et al. 2000 and Guo et al. 2004) with coefficient of variation (CV) for nutrients and fine root biomass using the UNIVARIATE procedure of SAS version 8 (SAS institute, Cary, North Carolina). I did not include sites with 20 sampling points because these sites had far less precise estimates of CVs and were not comparable to sites with 80 sampling points.

I used semivariogram analyses to determine spatial structure (Robertson and Gross 1994) of soil nutrients and fine root biomass at sites D1, S1, and F1. Semivariograms may reveal spatial patterns in nutrients at a scale that is relevant to individual plants. In a spatial dataset, the average variance of a group of sampling points at a certain "lag" distance is called semivariance (Isaaks and Srivastava 1989). The comparison of semivariance among different lag distances can be used to generate a semivariogram, where semivariance will generally increase with distance to a threshold level. This is because sampling points that are closer together are generally autocorrelated (i.e. have similar values), whereas points further apart are typically dissimilar. The semivariogram can be fitted with regression models (e.g. spherical, linear, exponential), and several statistics can be generated (Figure 2.2). The sill is the threshold where semivariance no longer increases with distance, and the range is the distance from zero where the sill occurs. The nugget is the amount of variance that

occurs at a scale smaller than the level of field sampling. The data will exhibit a "nugget" effect if the semivariogram does not exhibit any autocorrelation (Isaaks and Srivastava 1989). The semivariogram range was used to indicate the spatial pattern of variability; SH% (100 * (sill-nugget)/sill) and R^2 (amount of variation explained by the semivariogram model) was used to measure the degree of spatial structure (Rossi et al. 1992, Robertson and Gross 1994, Li and Reynolds 1995).

Semivariogram analyses require that the data are stationary (i.e. lack a spatial trend across the plot). Trends may mask fine-scale spatial structure that may be ecologically important if included in the semivariogram analysis (Gallardo 2003). Therefore, I used trend surface analysis (TSA) to detect and remove trends (Davis 1986). When spatial trends were detected, I used the residuals generated from TSA for semivariogram analyses.

Semivariogram analyses were performed using GS+ software version 5.1 (Gamma Design, Plainwell, Michigan). I selected lag distances based on a balance of equal lag distances and equal number of pairs for each lag distance (Zheng and Silliman 2000). I generated only non-directional (anisotropic) semivariograms because there was an insufficient number of sample pairs per lag distance to produce directional (isotropic) semivariograms. Nutrient and fine root biomass data were log transformed prior to semivariogram analyses when data were not normally distributed.

Hypothesis 3: Fine root biomass and soil nutrients within ecosystems

I used Spearman rank correlations to determine relationships between soil fertility and fine roots within sites. I ran separate correlations for each type of nutrient (NO_3-N, NH_4-N, and PO_4-P) for each site and calculated correlations between a nutrient index and fine root biomass. The nutrient index for each site was similar to that created for comparisons among sites, except that I separately ranked soil nutrients (NO_3-N, NH_4-N, and PO_4-P) for each sampling point within a site. A sequential Bonferroni correction was used to adjust alpha for the four correlations calculated at each site. I incorporated all 80 sampling points for sites D1, S1, and F1 for each correlation. In addition, I ran correlations between nutrient residuals generated from TSA and fine root biomass at

these sites to reveal potential relationships between these parameters that may have been masked by broad-scale spatial trends in nutrients across the site.

Results

Hypothesis 1: Ecosystem fertility and fine root biomass

Soil nutrient availability was different among wetland types (MANOVA, Wilk's lambda, $F_{6,8} = 3.67$, $p < 0.05$, Figure 2.3). However, this difference was driven by differences in mean NO_3-N among wetland types (ANOVA, $F_{2,6} = 10.78$, $p < 0.01$). Mean NO_3-N followed the expected trend: floodplain swamp > river swamp slough > depressional swamp. NO_3-N was ~2.5× greater at floodplain swamps compared to depressional swamps ($p < 0.01$); river swamp sloughs were intermediate. Wetland types did not differ in soil NH_4-N (ANOVA, $F_{2,6} = 0.40$, $p > 0.60$) or PO_4-P (ANOVA, $F_{2,6} = 2.73$, $p > 0.10$). Fine root biomass was ~2.5 to 3× lower in floodplain swamps than in depressional swamps or river swamp sloughs (ANOVA, $F_{2,6} = 2.73, 10.57$, $p < 0.05$, Figure 2.3). There was no difference in fine root biomass between river swamp sloughs and depressional swamps.

The multiple regression model with NO_3-N, NH_4-N, and PO_4-P as independent variables explained 98% of variation in fine root biomass among sites and was highly significant ($p < 0.001$). In addition, when the three types of soil nutrients (NO_3-N, NH_4-N, and PO_4-P) were combined into a nutrient index, there was a negative correlation between the nutrient index and fine root biomass (Spearman rank correlation, $R = -0.90$, $p < 0.05$, Figure 2.4). Although not significant, fine root biomass also was negatively correlated with NO_3-N ($R = -0.60$, $p = 0.09$) and PO_4-P ($R = -0.58$, $p = 0.10$).

There was some overlap in fertility among wetland types that I speculate can be explained by land use histories of individual sites. S2 was part of a horse corral in the early to mid 1900s. Grazing by horses may have increases sediment inputs from the surrounding uplands into the wetland and increased soil fertility. This effect may have been exacerbated by the lack of best management practices (BMPs) during this time (Craft and Casey 2000). Soil fertility of F1 may have been lower than F2 and F3 because it was isolated from row-crop agriculture, whereas both F2 and F3 may have received nutrient laden sediments from nearby row-crop activities.

Hypothesis 2: Nutrient heterogeneity

CV for NO_3-N at F1 and D1 was >1.4× greater than at S1 (Figure 2.5). Similarly, CV for NH_4-N was greatest at F1 followed by D2 and S1. CV for PO_4-P, in contrast, was highest at S1 and lowest at D1. Overall, CV for fine roots was much lower than CVs for nutrients at each site, but showed a similar pattern to that of CVs for NO_3-N and NH_4-N.

Nutrients and fine roots varied in their spatial structure at the three representative sites (D1, S1, and F1, Table 2.2). Trend surface analysis showed either significant 1st or 2nd order trends for NO_3-N and fine roots at all sites ($p < 0.05$) and significant 1st order trends for the nutrient index at sites D1 and S1 ($p < 0.05$). In addition, a linear model fit PO_4-P and detrended NO_3-N values at site F1.

Hypothesis 3: Fine root biomass and soil nutrients within ecosystems

None of the Spearman rank correlations between fine root biomass and soil nutrients (NO_3-N, NH_4-N, PO_4-P) or total nutrient index were significant ($p > 0.05$, data not shown). Neither were there any significant correlations between fine root biomass and nutrient residuals generated from trend surface analysis at sites D1, S1, and F1.

Discussion

Hypothesis 1: Ecosystem fertility and fine root biomass

My predictions that ecosystem fertility would be greater in alluvial swamps compared to non-alluvial swamps and that fine biomass is inversely related to ecosystem fertility were partially supported. Ecosystem fertility followed the expected trend (floodplain swamp > river swamp slough > depressional swamp) for soil NO_3-N but not for NH_4-N and PO_4-P. NO_3-N was greatest in the floodplain swamps and lowest in the depressional swamps, but soil NH_4-N and PO_4-P were not different among wetland types. Groundwater from the Floridan aquifer in the Dougherty Plain of southwestern Georgia, which ranges from 20 to 5000 μg/L NO_3-N, may provide NO_3-N inputs to floodplain swamps (S. P. Opsahl, personal communication, Joseph W. Jones Ecological Research Center, Newton, Georgia). However, in the same sites as ours, Craft and Casey (2000) found that soil N accumulation was not different between depressional swamps and river

swamp sloughs, whereas P accumulation was greater in river swamp sloughs than in the depressional swamps. High accumulations do not necessarily equate to greater availability. In depressional swamps, soil N and P were largely in recalcitrant organic forms that are unavailable to plants (Craft and Chiang 2002).

Fine root biomass was negatively related to nutrient availability. These results are consistent with other studies in forest ecosystems (Keyes and Grier 1981, Aber et al. 1985, Nadelhoffer et al. 1985, Vogt et al. 1987). Fine root biomass was lowest in the floodplain swamps, but it did not follow the expected pattern (depressional swamps > river swamp sloughs > floodplain swamps) because depressional swamps and river swamp sloughs did not differ. However, two river swamp sloughs (S1 and S3) were not inundated during the study year, and these sites had the greatest fine root biomass. Therefore, more carbon may have been allocated to fine roots to obtain water during drought conditions rather than responding to soil nutrient availability. Megonigal and Day (1992) showed that *T. distichum* saplings grown in periodically flooded mesocosms had higher root to shoot ratios and deeper roots systems than in continuously flooded mesocosms, presumably to access water and nutrients 50-60 cm below the soil surface. In addition, Baker et al. (2001) observed that fine root biomass was greater on well-drained soils than on poorly-drained soils in the Coosewhatchee River floodplain in South Carolina. Alternatively, the lack of flooding may have slowed root turnover rates at S1 and S3 leading to a relatively greater fine root standing crop. Fluctuations in the water table in the rooting zone can cause a change between anoxic and well-oxidized conditions and increase root turnover (Keeley 1979, Hook 1984, Kozlowski 1984, Jones et al. 1996). However, I observed that the rooting zone was well-oxidized during the study year at these sites. If only the depressional swamps and floodplain swamps were considered, NO_3-N was greater in the floodplain swamps than in the depressional swamps, and fine root biomass was greater in the depressional swamps than in the floodplain swamps. These data suggest that soil nutrient availability at the ecosystem scale may be an important factor affecting fine root biomass in my systems.

Hypothesis 2: Nutrient heterogeneity

I hypothesized that soil nutrient availability would be spatially patchy and predicted that the level of nutrient heterogeneity would follow the trend: floodplain swamp > river swamp slough > depressional swamp. My data partially support this prediction. Global variability of NO_3-N and NH_4-N was greatest in the floodplain swamp (F1) but was greater in the depressional swamp (D1) than in the river swamp slough (S1). Except for PO_4-P at S1, nutrient CVs were generally much lower than reported for other forest ecosystems (Lister et al. 2000, Gallardo 2003), suggesting that overall variability was relatively low at my sites. Therefore, differences in absolute nutrient variability among my sites may be minor.

In contrast with my prediction, nutrients did not appear to be patchy at scales relevant to individual plants. My sites were generally mature or maturing forests where roots may have fully exploited soil nutrients resulting in a relatively uniform depletion of soil nutrients. In fact, the CV of fine roots at my sites was <50% and was always lower than that for nutrient variability (Figure 2.5). Guo et al. (2003) found that the development of vegetation greatly decreased nutrient variability in pine forests of southeastern United States that had been recently clearcut or girdled. Rather than fine-scale patches, my sites mostly exhibited large-scale trends in soil nutrients across each site. Gallardo (2003) showed that the distance from a pond caused large-scale differences in certain soil properties within a floodplain forest in northwest Spain. Thus, the large-scale trends in soil nutrients at my sites may have been related to the direction of flooding.

It is possible that fine-scale spatial structure occurred, but I failed to detect it using my sampling regime. Even though many of my sampling points were 0.5 m apart, the smallest lag distance averaged ~2 m, which may have been too large to detect patterns. Jackson and Caldwell (1993a, 1993b) observed that nitrogen and phosphorus were strongly autocorrelated at distances less than a meter but not at distances greater than a meter in a sagebrush-steppe ecosystem in Utah. In forest ecosystems, Lechowicz and Bell (1991) observed autocorrelation up to only 2 m for soil pH and NO_3-N, and Palmer (1990) showed autocorrelation within 1 m for soil P. However, in a pine forest in southeastern United States, Lister et al. (2000) showed autocorrelation at scales of >10 m

for NO_3-N, and Guo (2003) found no spatial dependence in soil N or P or very large autocorrelation ranges of 18 - 47 m. Thus, my sampling may also have been too fine to capture trends that were larger in scale.

Hypothesis 3: Fine root biomass and soil nutrients within ecosystems

My data did not support the hypothesis that nutrients and fine roots are positively correlated at fine spatial scales as correlations within sites were weak. Other studies, however, have demonstrated strong correlations between fine root biomass and nutrient-rich microsites (St. John et al. 1983, Fahey and Hughes 1994, Mordelet et al. 1996). Mou et al. (1995) suggested that this correlation may be nutrient specific, because they found a positive correlation between root density and soil P and K, but not soil N.

I propose three possibilities to explain why fine root biomass and soil nutrients were either unrelated or poorly correlated. First, nutrients extracted from ion exchange membranes may have not have been a good indicator of nutrients available to plants. Second, stresses associated with soil anoxia may prevent efficient root foraging for nutrients in wetlands. Hence, forested wetland species may be selectively placing roots in oxygen-rich patches rather than nutrient-rich patches to avoid stresses to root systems from soil anoxia. Jones et al. (1996) found that fine root biomass was greater in higher areas (oxygen-rich) than in lower areas (oxygen-poor), although the opposite pattern was found with fine root net primary production. However, I feel that stresses to root systems due to soil anoxia were low because my study sites were not saturated when roots and nutrients were measured. Furthermore, tree species in wetlands generally have adaptations to soil anoxia (e.g. water roots, aerenchyma) that allow root growth in flooded conditions. A third possibility for low root to nutrient correlation is the inadequacy of my nutrient sampling to capture long-term nutrient regimes. Nutrients vary temporally as well as spatially (Ehrenfeld 1997, Guo et al. 2004). I measured nutrient availability only once toward the end of the growing season. It is quite possible that nutrient conditions were different earlier in the growing season. Variation in fine root lifespan (Matamala et al. 2003, Trumbore and Gaudinski 2003) further reduces the ability to relate one-time measures of nutrient availability to one-time measures of fine root standing crop. In addition, fine root biomass may be relatively high in areas that

have become just recently depleted. There is often a local depletion of nutrients and water immediately adjacent to fine roots (Fitter and Hay 1987), and Van Vuuren et al. (1995) found that fine root proliferation into N-rich patches can continue even after nearly all of the N is gone.

Conclusion

My study suggests that ecosystem-scale fertility is more important than local-scale in determining the amount and distribution of fine root biomass in forested wetlands. I speculate that soil anoxia may be a critical driver of root responses that may mask influences of fine-scale nutrient heterogeneity. To test this idea, I suggest that future studies should consider fine-scale heterogeneity of both nutrient availability and oxygen in soil.

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Table 2.1 Basal area (m^2/ha) of trees (DBH > 2 cm) at the nine study sites, based on measures of diameter at 1.4 m (DBH), or above butt swell for *Nyssa* and *Taxodium* spp., made in one 20 × 20 m plot per site.

	Wetland type											
	Depressional swamp				River swamp slough				Floodplain swamp			
Species	D1	D2	D3	mean	S1	S2	S3	mean	F1	F2	F3	mean
Nyssa biflora	56.8	30.9	41.3	43.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Taxodium ascendens	15.0	48.5	26.0	29.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Taxodium distichum	0.0	0.0	0.0	0.0	19.4	48.6	15.3	27.8	4.2	0.0	6.6	3.6
Planera aquatica	0.0	0.0	0.0	0.0	14.3	0.0	27.6	14.0	1.6	2.4	0.0	1.3
Nyssa aquatica	0.0	0.0	0.0	0.0	3.8	75.0	0.0	26.3	25.9	75.7	61.4	54.3
Quercus laurifolia	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.5	3.7	5.2	0.0	3.0
Fraxinus pennsylvanica	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.6	3.6	0.0	5.4
Quercus lyrata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.3	0.4	0.0	1.9
Others	1.1	0.3	0.6	0.7	0.0	1.6	7.7	3.1	3.3	1.0	0.3	1.5
Total	72.9	79.7	67.9	73.5	37.6	126.7	50.6	71.6	56.6	88.4	68.3	71.1

Table 2.2 Summary of trend surface and semivariogram analyses for D1, S1, and F1 (D = depressional swamp, S = river swamp slough, F = floodplain swamp). Trends were either 1st or 2nd order with level of significance indicated by: *<0.05, **<0.01, and ***<0.001. A lack of trend is indicated by --. Semivariogram parameters (range, SH%, and R^2 values are shown for each site. The best model based on least squares (L=linear, N=nugget) are shown after R^2 values. When a nugget was found, all 3 semivariograms parameters are indicated with '--' because the parameters are undefined. The range for linear models is equal to the maximum lag distance because the model never reaches sill.

Plot	Parameter	NO_3-N	NH_4-N	PO_4-P	Nutrient index	Fine roots
D1	Trend	1st**	--	--	1st**	1st*
	Range	--	--	--	--	--
	SH%	--	--	--	--	--
	R^2/Model	N	N	N	N	N
S1	Trend	1st***	--	--	1st***	2nd***
	Range	--	--	--	--	--
	SH%	--	--	--	--	--
	R^2/Model	N	N	N	N	N
F1	Trend	2nd***	--	--	--	1st*
	Range	23.3	--	23.3	--	--
	SH%	71	--	69	--	--
	R^2/Model	0.54/L	N	0.42/L	N	N

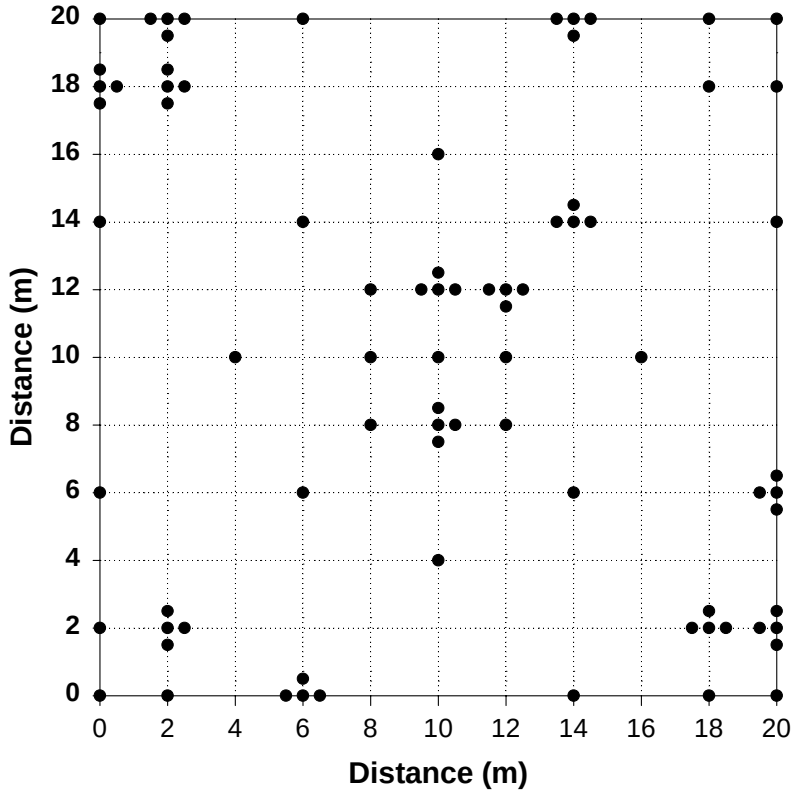

Figure 2.1 Sampling design at sites D1, S1, and F1. In each plot, 41 points were located at intersections of a 2 × 2-m grid. An additional 39 points were arrayed in sets of 3 around 13 randomly selected points at grid intersections. The sets of 3 points were randomly oriented in a north, south, east, or west direction and each point was located 0.5 m from a grid intersection. The sampling design optimized the number of lag classes for semivariogram analyses where the minimum sampling interval was 0.5 m.

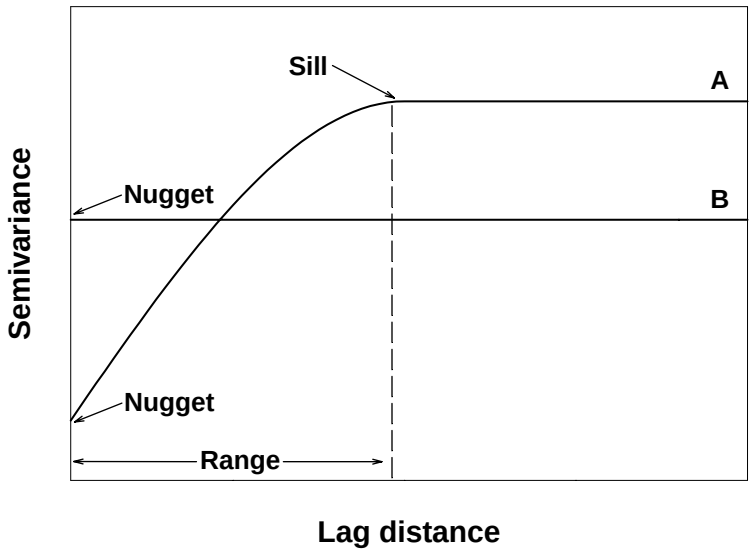

Figure 2.2 Theoretical example of a semivariogram that shows semivariance as lag distance increases. Curve A is a spherical model where the semivariogram exhibits spatial autocorrelation over a certain range and independence thereafter. Curve B is a "nugget effect" where the semivariogram does not show any spatial autocorrelation.

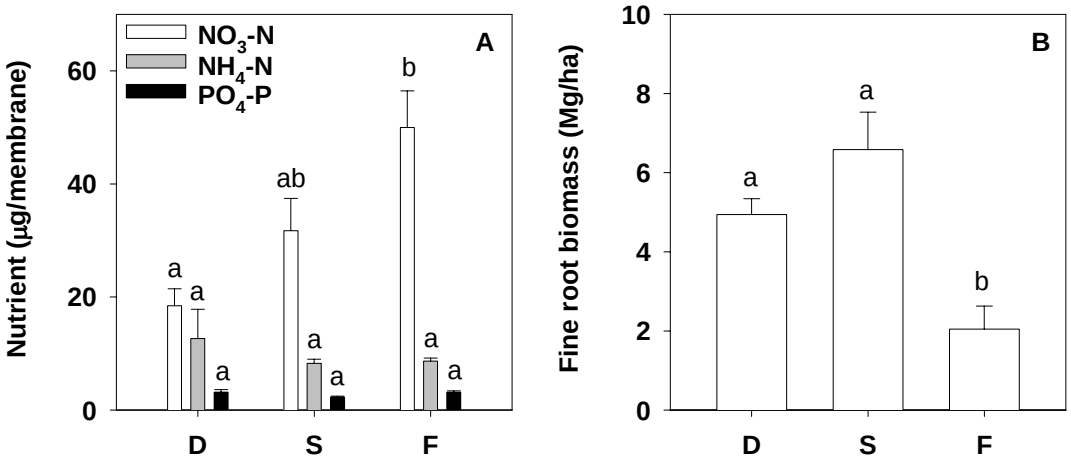

Figure 2.3 Soil nutrient availability (panel A) and root biomass (panel B) in three wetland types. D = depressional swamps, S = river swamp sloughs, and F = floodplain swamps. Different letters indicate significant differences ($p < 0.05$) among site types.

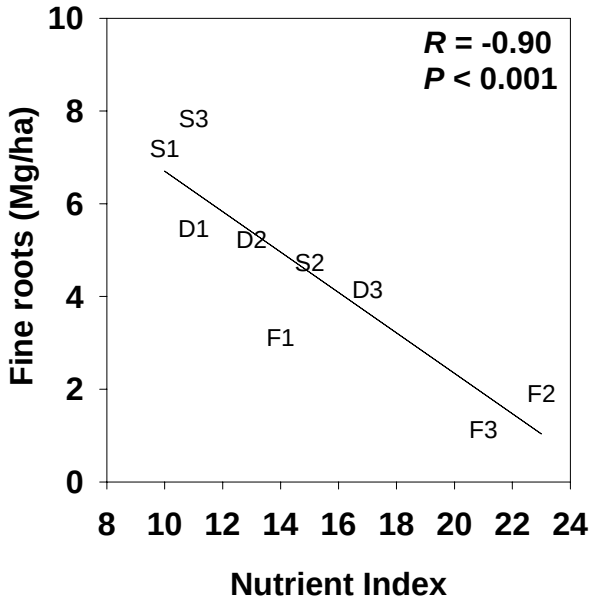

Figure 2.4 Relationship between nutrient index and fine root biomass based on Spearman rank correlation. Individual sites are indicated in each panel, D = depresssional swamps, S = river swamp sloughs, F = floodplain swamps. The nutrient index was obtained by ranking site means for each nutrient among all sites and summing the ranks.

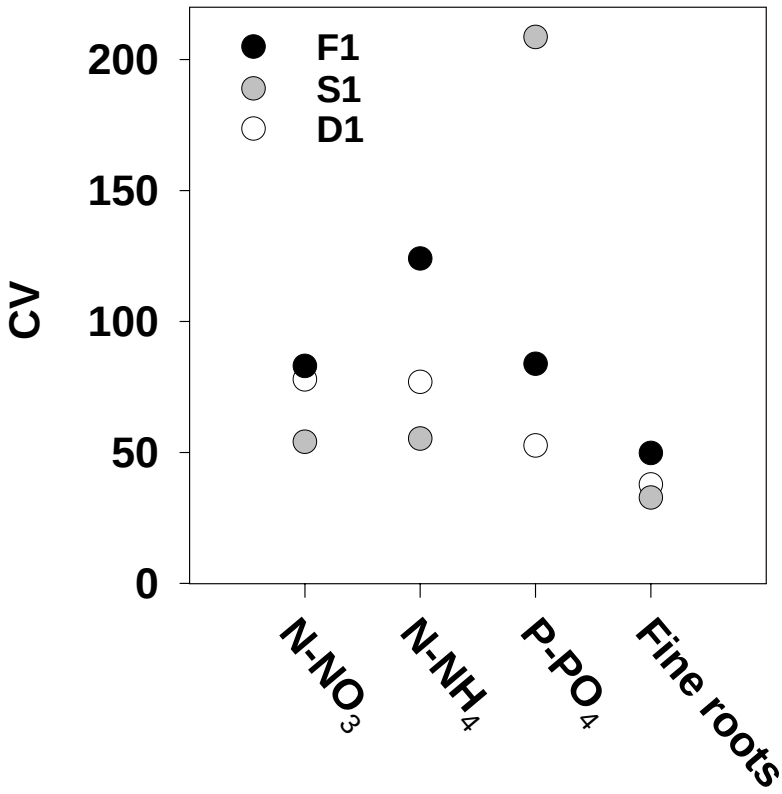

Figure 2.5 Coefficient of variation (CV) of NO_3-N, NH_4-N, PO_4-P, and fine root biomass at sites D1, S1, and F1 (D = depressional swamp, S = river swamp slough, F = floodplain swamp). Panel A = variance scaled to 1. Panel B = coefficient of variation (CV).

Chapter 3. Root system response to microsite P enrichment in forested wetlands with contrasting hydrologic regimes

Abstract

I hypothesized that root proliferation in nutrient-rich patches would be more pronounced in fertile floodplain swamps (F) compared to infertile depressional swamps (D). To test this hypothesis, I created nutrient-rich microsites within three depressional and three floodplain swamps by fertilizing ingrowth cores containing sand with different levels of phosphorus (P): 0, 2, 4, 8× ambient levels. Fertilized ingrowth cores containing native soil were used at one site (D1) to check the effectiveness of sand as a growth medium. Root growth was positively related to the amount of P in a microsite at only two sites: D1 with native soil and F1. However, there was no response to P enrichment at D1 when ingrowth cores contained sand instead of native soil. There was no difference in root system response to P patches between floodplain and depressional swamps. My data are inconclusive because sand is probably a poor growth medium for forested wetland species and suggest that future studies should use native soil in ingrowth cores rather than sand.

Introduction

Resource availability in ecosystems exerts strong selection pressure on plants and may guide the evolution of certain plant traits (Chapin et al. 1993). To reduce the demand for and conserve nutrients, plants adapted to nutrient-poor conditions typically have low relative growth and nutrient uptake rates, long tissue lifespan, high root:shoot ratios, and high amounts of defense compounds (Grime 1977, Chapin 1980, Eissenstat and Yanai 1997). In contrast, plants adapted to nutrient-rich conditions generally have the opposite characteristics.

Plant adaptive strategies to nutrient-poor or nutrient-rich ecosystems may affect how root systems respond to nutrient heterogeneity. It is well established that plants can respond to soil nutrient heterogeneity by proliferating roots into nutrient-rich patches (Robinson 1994, Hodge 2004). However, species often differ in the magnitude of this response (e.g. Crick and Grime 1987, Einsmann 1999) and this may be at least partly related to adaptations to nutrient-poor or nutrient-rich ecosystems. Empirical studies have shown that plants adapted to nutrient-rich conditions typically respond more via root proliferation to nutrient-rich patches than do plants adapted to nutrient-poor conditions (Crick and Grime 1987, Hutchings and de Kroon 1994, Fransen et al. 1998, 1999, but see Aanderud et al. 2003). However, these studies have largely focused on response of individual seedlings grown in pots within a greenhouse. Few studies have attempted to study root system response to nutrient heterogeneity in natural ecosystems.

Root system response to nutrient heterogeneity in forested wetlands of the southeastern United States may be affected by hydrologic regime because flooding can influence both the spatial and temporal availability of nutrients. For example, Fertility is greater in alluvial or floodplain swamps that receive external inputs of nutrients from precipitation and river flooding compared to non-alluvial or depressional swamps that obtain nutrients via precipitation alone. In this study, I investigated how root systems respond to nutrient heterogeneity in depressional and floodplain swamp ecosystems. I created small-scale phosphorus (P) patches by enriching root ingrowth cores with phosphorus. I predicted that root proliferation would be positively related to the amount of P in a patch. In addition, I expected that that root system response to P patches would be more pronounced in floodplain swamps than in depressional swamps.

Methods

Study sites

This study was conducted from July to November 2002 in depressional swamps (D) at the Joseph W. Jones Ecological Research Center in Baker County, Georgia and floodplain swamps (F) at the Chickasawatchee Wildlife Management Area in Baker and Calhoun counties, Georgia. Three 20×20 m plots were established in each swamp type. During the study year (January 2003 - December 2003), mean temperature was 18ºC and annual precipitation was 121 cm, which was 10% below normal (National Climatic Data Center, Asheville, North Carolina). Soils in depressional swamps have an organic-rich A horizon and were classified as Histic Humaquepts, whereas soils in floodplain swamp were classified as Typic Fluvaquents of the Muckalle series. *Nyssa biflora* Walt. and *Taxodium ascendens* Brong. were dominants in the overstory of depressional swamps. In floodplain swamps, the overstory consisted mainly of *Nyssa aquatica* L., *Fraxinus pennsylvanica* Marsh., *Quercus laurifolia* Michx., *Quercus lyrata* Walt., and *Taxodium distichum* (L.) Rich.

Field and lab measurements

Root ingrowth cores (Oliveira et al. 2000) were installed to determine root response to P-rich patches. Phosphorus was manipulated because previous research has suggested that these swamps are P-limited (Watt and Golladay 1999, Craft and Casey 2000). A preliminary study was used to determine how much fertilizer was needed to geometrically raise P availability by 1, 2, 4, and 8× ambient levels. In one depressional swamp and one floodplain swamp, ingrowth cores were installed containing play sand mixed with 0 to 40 g of ammonium phosphate (10-48-0 NPK, Harrells Inc., Lakeland, Florida). Play sand is washed to remove silt and clay and consists of 14% coarse sand (0.5-1.0 mm), 48% medium sand (0.25-0.5 mm), 36% fine sand (0.10 – 0.25 mm), and 1% very fine sand (0.05-0.10 mm) (The Quikrete Companies, Atlanta, Georgia). Also, I installed three additional ingrowth cores per site containing native soil to measure ambient P availability. I placed three 2 × 4.5-cm strips of ion exchange membranes (type 204-UZR-456 for PO_4, Ionics Inc., Watertown, Massachusetts) into each core. Membranes were charged with 0.5 M $NaHCO_3$ prior to installation. After 72 h, I

removed the membranes from the core, extracted them with 0.5 M HCl, and measured PO_4-P on an autoanalyzer (Lachat Quickchem AE, Lachat Instruments, Milwaukee, Wisconsin).

P availability, as measured by membranes, was similar in ingrowth cores containing native soil to that of cores containing sand that was not fertilized at both sites. Thus, adding no fertilizer to the core was considered to be equivalent to ambient P availability. Because membrane P availability was similar at each site for each fertilizer level, I combined data from both sites and constructed a regression line with fertilizer level as the independent variable and P availability as the dependent variable. I used the regression to determine how much fertilizer was needed to raise P availability by 1, 2, 4, and 8× ambient levels, which corresponded to 0, 0.75, 1.93, and 4.30 g of ammonia phosphate.

Within each site, I randomly removed twenty 10.16-cm diameter x 20-cm deep soil cores in July 2002. Holes were replaced with play sand that was randomly assigned to one of the four P treatments. Although I intended to only manipulate P availability, I realize that nitrogen availability was also manipulated to a lesser extent because the fertilizer was 10% ammoniacal nitrogen. Each treatment was replicated five times per site.

In November 2002, I extracted a 7.62-cm diameter x 20-cm deep soil core from the middle of each ingrowth core and washed the core over a 1-mm mesh screen to remove adhering sand from roots. Roots were separated into fine (< 2 mm) and coarse (> 2 mm) size classes. One subsample of roots from each core was placed over a 2 cm^2 grid to determine root length density (km root per m^3 of soil) using the line intersect method (Tennant 1975). All samples and subsamples were dried at 60ºC to constant weight and weighed.

Data analysis

I ran a simple linear regression for each site to determine whether root proliferation was positively related to the amount of P in a patch. Each ingrowth core was treated as a replicate. Alpha was adjusted using a sequential Bonferroni technique to avoid inflation of the Type I error rate. A general linear model with an indicator variable

was used in SAS version 9 (GLM procedure, SAS institute, Cary, North Carolina) to determine whether swamp types (i.e. depressional vs. floodplain) responded differently to P-enrichment. Sites were replicates in this analysis.

Results

Fine root mass and length densities were positively related to nutrient enrichment only at D1 when native soil was fertilized and F1 (Figure 3.1, 3.2). In addition, fine root response to P patches was not different between depressional and floodplain swamps for either fine root mass density ($p = 0.13$, Figure 3.3) or fine root length density ($p = 0.15$). The lack of difference between swamp types was probably due to different responses of each individual site to nutrient enrichment (Figure 3.4).

Discussion

My hypothesis that root proliferation would be positively related to the amount of P in patch within each site was not supported. In fact, only two sites showed a significant response to microsite P enrichment, and ingrowth cores at one of these sites (D1) contained native soil instead of play sand. Also, my second hypothesis that root response to nutrient-rich patches would be different between depressional and floodplain swamps was not supported.

These findings contradict the results of other studies conducted in forest ecosystems. In a mixed hardwood forest in Michigan, Pregitzer et al. (1993) found new root production into water + nitrogen patches was much greater compared to water patches alone. In addition, St. John (1983) observed greater root growth into mesh bags containing leaf litter and sand compared to bags filled only with sand. Leaves were presumably a source of mineralized nutrients.

In my study, root systems may have not have not the genetic capability to respond morphologically to nutrient-rich patches. However, this is unlikely because species common to these ecosystems (*Fraxinus pennsylvanica* and *Nyssa aquatica*) were found to proliferate roots into nutrient-rich patches in a pot study (see Chapter 5). Rather, play sand was most likely a poor growth medium for roots. At D1, root growth was positively related to the level of P in a patch when native soil was used in ingrowth cores, but not

when play sand was used alone. Lack of water, organic matter, or some critical nutrient (e.g. potassium) may have limited root growth into the play sand. In addition, P may have leached out of the cores. These data suggest that experiments with root ingrowth cores should contain native soil or at least a growth medium containing some organic matter to better simulate soil conditions in the native soil.

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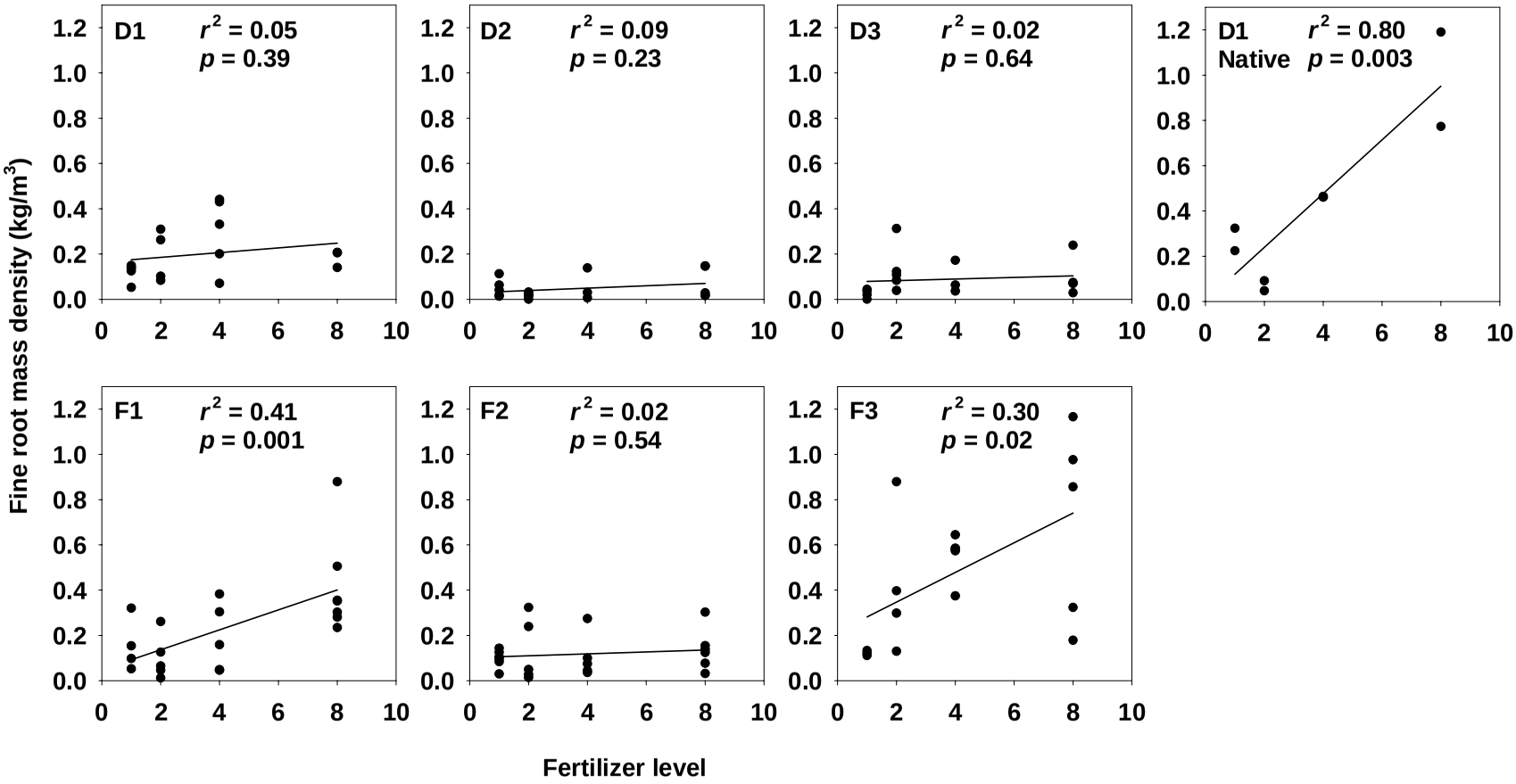

Figure 3.1 Response of fine roots (fine root mass density) to different levels of small-scale nutrient enrichment within depressional and floodplain swamps. Fertilizer level is the multiple (1, 2, 4, and 8×) above ambient P availability, which corresponds to 0, 0.74, 1.93, and 4.30 g of ammonia phosphate added to play sand in root ingrowth cores. Fertilizer was also added to ingrowth cores filled with native soil at one site (D1).

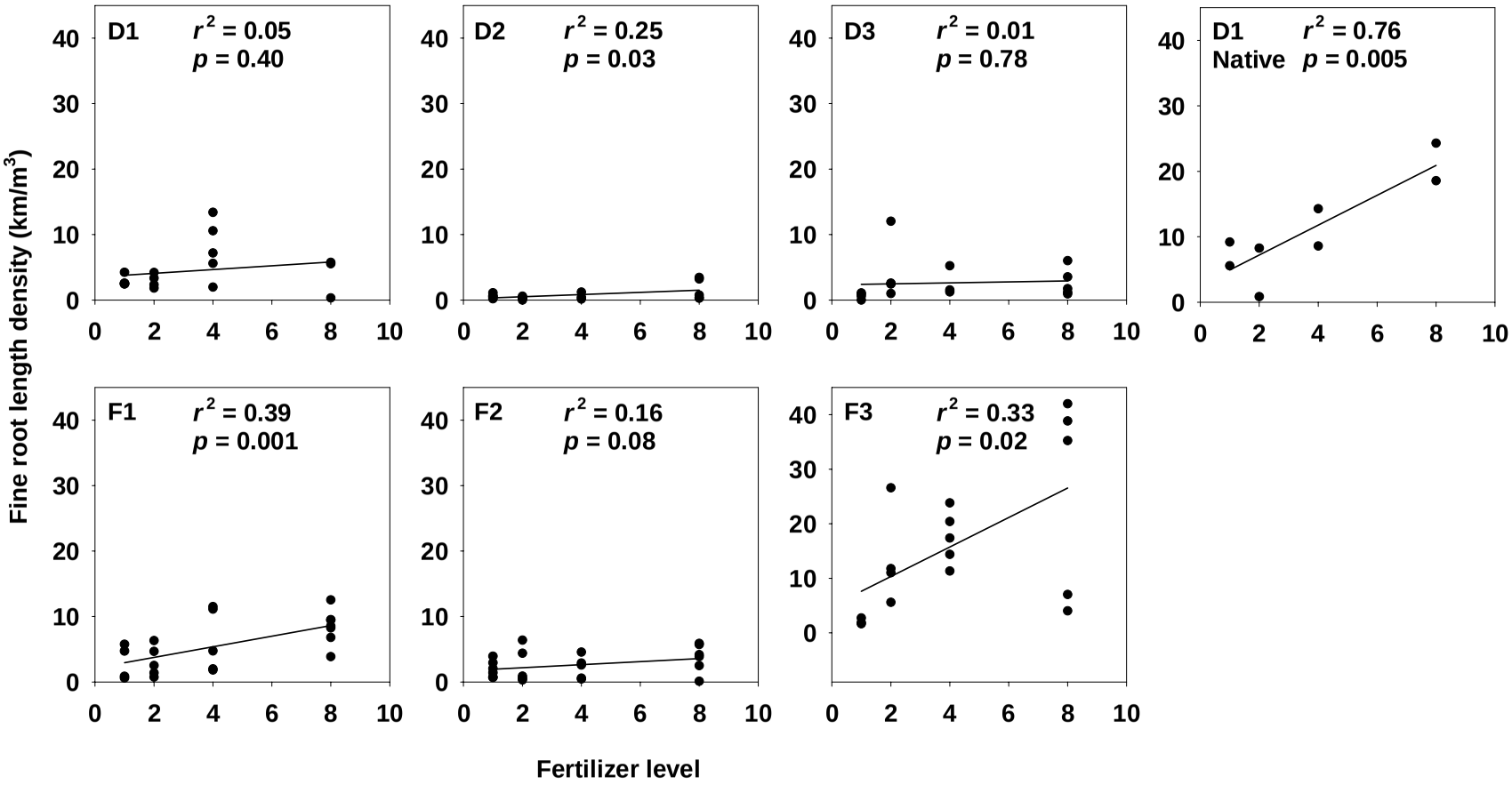

Figure 3.2 Response of fine roots (fine root length density) to different levels of small-scale nutrient enrichment within depressional and floodplain swamps. Fertilizer level is the multiple (1, 2, 4, and 8×) above ambient P availability, which corresponds to 0, 0.74, 1.93, and 4.30 g of ammonia phosphate added to play sand in root ingrowth cores. Fertilizer was also added to ingrowth cores filled with native soil at one site (D1).

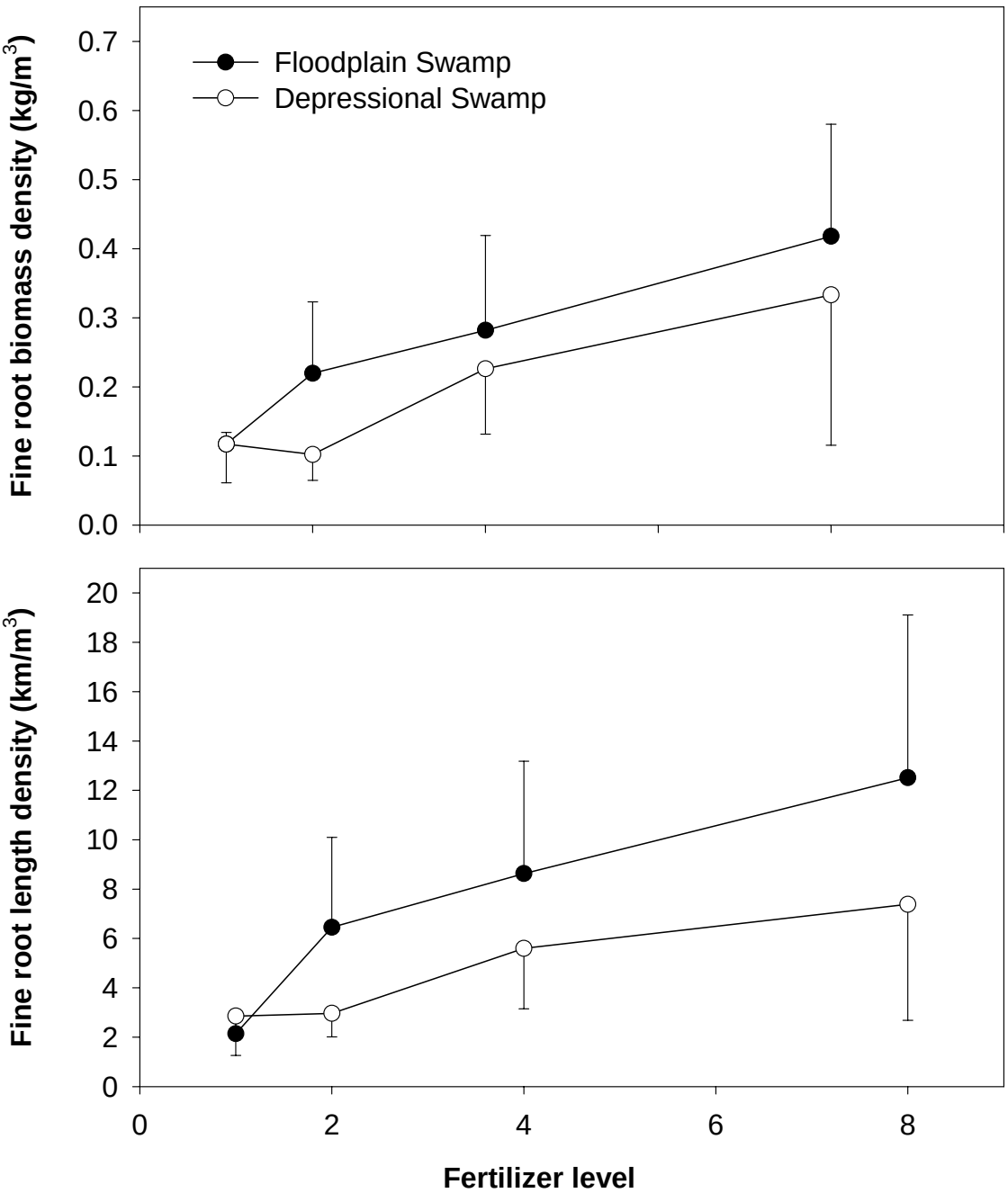

Figure 3.3 Comparison of fine root response to nutrient patches between depressional and floodplain swamps.

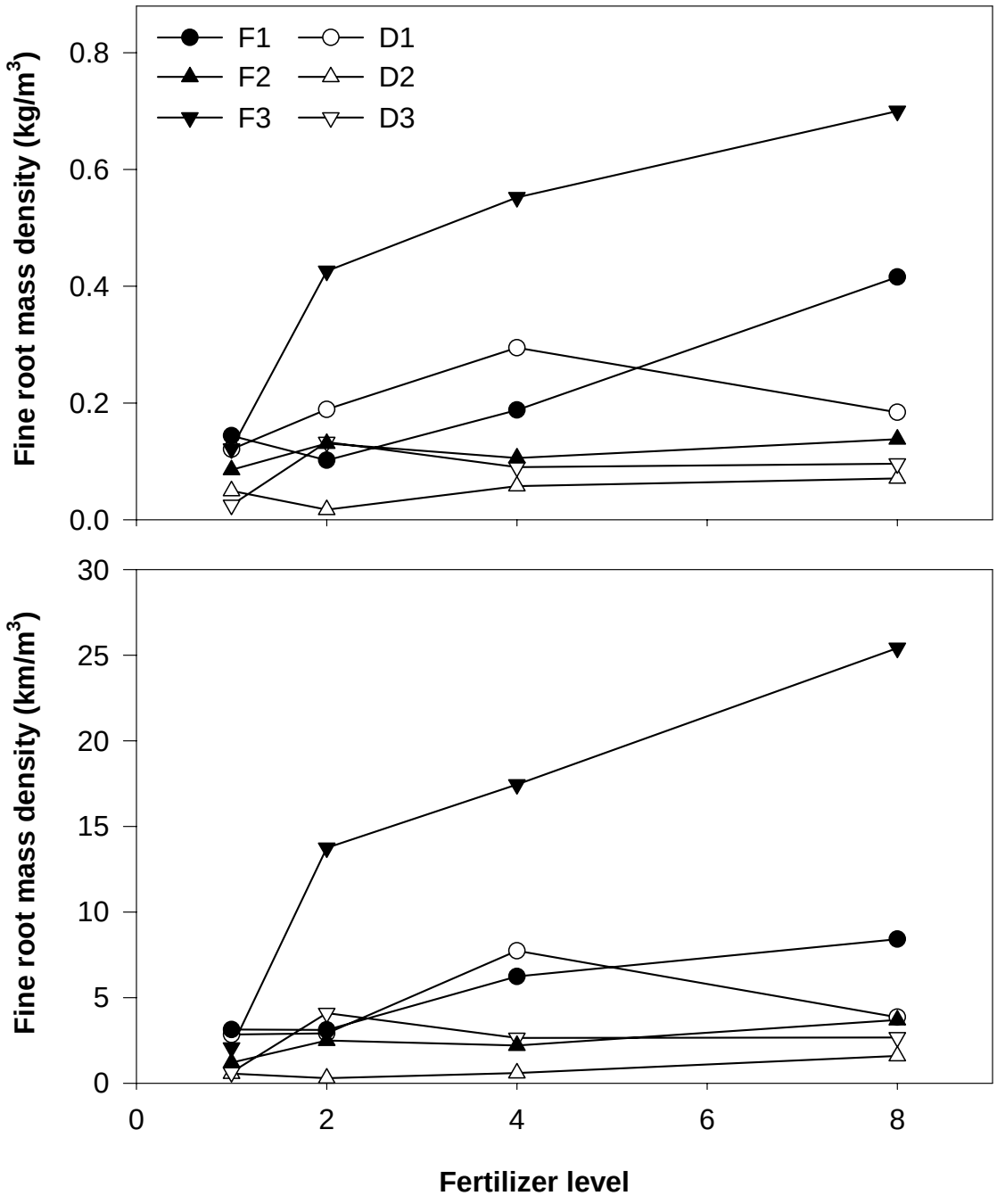

Figure 3.4 Mean root response to nutrient-patches at each individual site.

Chapter 4. Relationship between ecosystem nutrient limitation and fine root foraging for soil nutrients in a floodplain forest in southern Georgia, USA

Abstract

I explored relationships between ecosystem-level nitrogen and phosphorus limitations and fine root foraging behavior in a floodplain forest in southern Georgia, USA. I predicted that root proliferation would be greatest in microsites that were rich in the ecosystem's most limiting nutrient. Root ingrowth cores were installed in three replicate sites and were either not fertilized (control) or fertilized with nitrogen (N), phosphorus (P), or nitrogen and phosphorus (N+P). I collected plant litter using litterfall traps to measure litter N and P content. N:P ratios in plant litter were used to determine whether N or P was limiting. Fine roots responded to microsite N fertilization at two sites but not to P fertilization. However, litter N:P ratio ranged from 18 to 24 indicating strong P limitation. I propose three reasons why roots responded to the apparently less limiting nutrient. First, P in fertilizer may have been unavailable to plants due P sorption into aluminum and iron complexes. Second, plants may have not had the genetic capability to respond to P. Third, N may have been limiting, suggesting that litter N:P ratios may be a poor indicator of whether N or P is limiting in these wetlands.

Introduction

In most forest ecosystems, plant growth is limited by the availability of soil phosphorus or nitrogen (Aerts and Chapin 2000), but it is not known whether ecosystem nutrient limitation affects how plants forage for nutrients. Soil nutrients are typically heterogeneously distributed within the rooting zone of individual plants (Jackson and Caldwell 1993a, 1993b, Lechowicz and Bell 1991, Ehrenfeld et al. 1997). Plants can respond when nutrient-rich patches are encountered by altering root system morphology via a change in root architecture (Fitter et al. 1991) or proliferation of root mass or length within nutrient-rich patches (e.g. Drew 1975, Drew and Saker 1978, Mou et al. 1997, Mordelet et al. 1996, Einsmann et al. 1999).

Different types of nutrients will often elicit different degrees of root proliferation (Drew 1975, Drew and Saker 1978, Jackson and Caldwell 1989). For example, Drew (1975) found that *Hordeum vulgare* L. increased root growth when exposed to localized patches of phosphate, nitrate, and ammonium, but not to potassium. There are at least two hypotheses why plants may respond differently to different types of nutrients. First, plants may be adapted to forage only for specific nutrients regardless of environmental conditions. Haines (2002) showed that root proliferation of *Thlaspi caerulescens* J. and C. Presl. in zinc-rich patches was greater in an ecotype from a site heavily contaminated with zinc than an ecotype from a less contaminated site. Alternatively, root proliferation may be a general response to limiting nutrients, with the response dependent on plant nutritional status (Robinson 1994, Jackson and Caldwell 1989, Hodge 2004), but this has never been explicitly tested. In *Liquidambar styraciflua* L. and *Pinus taeda* L. monocultures, Mou et al. (1995) found that the spatial distribution of fine root biomass was correlated with soil phosphorus (P) and potassium (K) but not nitrogen (N). Although they suggested that P limitation may be responsible for the strong correlation between soil P and root biomass, they did not determine which nutrient was limiting. Knowing what controls fine root response to nutrient-rich patches may give key insight into ecosystem carbon allocation patterns. For example, proportionally more of total plant carbon may be allocated aboveground if plants can efficiently proliferate their roots into patches of limiting nutrients and avoid proliferation within patches dominated by non-limiting nutrients.

Koerselman and Meuleman (1996) proposed that N:P ratio in plant tissue can generally be used to indicate whether N or P is limiting within an ecosystem. However, Aerts and Chapin (2000) cautioned that N:P ratios may be meaningless if another nutrient besides N or P is limiting. Koerselman and Meuleman suggested that N:P ratio > 16 indicated P limitation, whereas N:P ratio < 14 was N limitation. Ecosystems with N:P ratios between 14 and 16 were co-limited by N and P. In forested wetland ecosystems, Lockaby and Conner (1999) suggested that forests with litterfall N:P ratios >15 were P-limited, whereas those with N:P ratios <15 were N-limited.

Floodplain forests adjacent to blackwater streams within the coastal plain are often highly productive but typically low in soil N and P relative to other floodplain forests. Thus, precise root proliferation into nutrient-rich patches may be particularly advantageous and strongly expressed. Here, I investigated whether ecosystem nutrient limitation influences fine root foraging in a blackwater, floodplain forest in the Gulf Coastal Plain of southern Georgia, USA. N:P ratios in litterfall were measured to determine whether N or P limit forest productivity. Microsites with fertilized with N, P, or N+P to determine the degree of fine root response to different types of nutrients. I predicted that roots would be respond more to P-rich patches than N-rich patches if litterfall N:P ratios were > 15, whereas roots would respond more to N-rich patches than to P-rich patches if litterfall N:P ratios were < 15.

Methods

Site description

One 26×26 m plot was established in each of three sites (A, B, and C) within the floodplain of Chickasawhatchee Creek in the Chickasawhatchee Wildlife Management area of Baker and Calhoun counties, Georgia, USA. The floodplain is now second growth forest following logging in the first half of the 20th century. The overstory is dominated at all sites by various *Quercus* species (*Quercus lyrata* Walt., *Quercus laurifolia* Michx., *Quercus nigra* L), *Liquidambar styraciflua* L., and *Acer rubrum* L. At site B, *Pinus taeda* L. was also dominant. The understory consists primarily of *Smilax* spp., *Toxicodendron radicans* (L.) Kuntze and *Sabal minor* (Jacq.) Pers. Soils are classified as either Typic Alabaqualfs (Megget series) or Typic Fluvaquents (Muckalee

series) (USDA 1985, 1986). I observed that soils were saturated within the rooting zone (< 20 cm from surface) in spring and early summer 2003, but water table depth dropped to >20 cm from the surface by the beginning of my study (July 2003). Mean annual temperature during the study year was 19°C and annual precipitation was 141 cm, which is 11% above normal (National Climatic Data Center, Asheville, North Carolina).

Roots

I measured fine root response to microsite fertility using root ingrowth cores Oliveira et al. 2000). Each plot was divided into 2×2 m grid cells consisting of 196 grid intersections and randomly designated 44 of the intersections for installation of root ingrowth cores. In early July 2003, I extracted a 10.16-cm diameter × 20-cm deep hole at each core location. The core hole was replaced with soil of the same type collected from an adjacent site and sieved through a 2-mm screen to remove roots. Soil was packed to approximate the same bulk density as the soil that was removed. Ingrowth cores consisted of one of four treatments: 1) a control that was not fertilized (C); 2) phosphorus added only (P); 3) nitrogen added only (N); or 4) nitrogen + phosphorus added (N+P). Each treatment was replicated 11 times per plot per treatment. Nitrogen was added as 0.75 g of POLYON coated urea (43-0-0 NPK, Harrell's Inc., Lakeland, Florida), and phosphorus was added as 0.40 g triplesuperphosphate (0-46-0 NPK, Southern States Cooperative, Christiansburg, Virginia) to raise soil nutrient availability by 400 kg N/ha and 100 kg P/ha, respectively. Fertilizer levels were chosen because they are commonly used by foresters to overcome nutrient deficiencies in tree plantations in southeastern United States (Allen 1987). Fertilizer was mixed into the top 5 cm of each ingrowth core. In late November 2003, I extracted a 7.62-cm diameter × 20-cm deep core in the middle of each ingrowth core. Seven cores were not collected due to damage by animals. Roots were washed over 1-mm mesh screen to remove adhering soil particles. Samples were separated from soil organic matter in the lab and subsampled for root length analyses. Each subsample was scanned and analyzed using WinRHIZO software (Regent Instruments, Quebec, Canada) to determine specific root length (SRL, cm of root per g of root) and root length density (km root per m^3 of soil). Roots samples and subsamples were dried to a constant weight at 60˚C and weighed.

Litter nutrient content

I randomly placed five 0.25-m^2 litter traps within each plot in September 2003 and collected litterfall in November 2003 and May 2004. Litter was dried to a constant weight (60˚C), weighed, ground using a Wiley mill (Thomas Scientific, Swedesboro, New Jersey), and pulverized using a ball Spex 8000D ball grinder. Litter N content was determined using the dry combustion method with a Perkin Elmer Series II CHNS/O analyzer (Perkin Elmer Inc., Boston, Massachusetts). I used the dry ash method to determine litter P content (Chapman and Pratt 1961). Ashed samples were analyzed for P content with a Lachat Quickchem AE autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin) using the molybdate-blue method.

Soils

I collected and combined 40 2-cm diameter × 20-cm deep soil cores of mineral soil from each plot. Soil was sieved through a 2-mm screen to remove coarse fragments. Soil pH was determined using an Accumet pH meter and total N and C using a Perkin Elmer Series II CHNS/O analyzer. In addition, I performed an anaerobic incubation as an index of available N (Bundy and Meisinger 1994). Extractants were analyzed for NH_4-N on the Lachat Quickchem AE autoanalyzer. Extractable soil nutrients (P, K, Ca, Mg, and Fe) were determined using a Mehlich I extraction technique (Mehlich 1984) at the Virginia Tech Soil Testing Lab.

Data analyses

I tested the effect of microsite fertilization on fine root response using a nested design with fertilization treatments nested within sites. Separate ANOVAs were run with either fine root length, fine root biomass, or SRL as response variables using Proc Mixed (Statistical Analysis System, Cary, North Carolina). To measure the efficiency of internal cycling within trees, I determined nutrient use efficiency of both N and P. NUE is defined as the dry mass to nutrient content ratio in litterfall (Vitousek 1982). When NUE is high, internal cycling is efficient, which is expected in nutrient-poor ecosystems. Litterfall N and P content and N:P ratios were calculated by a weighted average based on litter mass in each trap.

Results

Soils were acidic, especially at site B (Table 4.1). Total C, total N, and extractable P, K, Mg, and Fe were similar among the sites. However, available N was much greater at site C than at sites A or B. In addition, soil Ca was much lower at site B than at sites A or C. Fine root length density (RLD) differed among the microsite fertilization treatments at two of the three sites (ANOVA, $F_{9,114} = 4.0$, $p < 0.001$) (Figure 4.1). Root length density was >2× greater at site A, and >1.7× greater at site C in N and N+P enriched microsites than in either C or P microsites (Tukeys test, $p < 0.01$). However, there were no differences in root length density between N and N+P microsites ($p > 0.42$) or between C and P microsites ($p > 0.56$). In addition, there were no differences among fertilization treatments at site B ($p > 0.09$). Fine root biomass responded to microsite fertilization ($F_{3,114} = 2.0$, $p < 0.05$) but only at site A where biomass was >2× in N and N+P microsites than in C or P microsites ($p < 0.05$). Root diameter was unaffected by treatment; no significant difference in SRL was found among the treatments ($F_{3,114} = 0.6$, $p = 0.64$). Litterfall N:P ratio ranged from 18 to 24, suggesting that P limits forest productivity (Table 4.2). Litterfall N and P concentrations ranged from 0.80% to 1.09 and 0.03% to 0.05%, respectively. N NUE ranged from 93 to 124 and P NUE ranged from 1950 to 2990.

Discussion

My hypothesis that fine root proliferation should be greatest in fertilized patches of the most limiting nutrient was not supported. Litterfall N:P ratios were >15 at each site indicating that P was limiting. Root proliferation, measured as root length density, was only augmented relative to the control when microsite N was enriched at sites A and C. In contrast, roots did not respond to P fertilization at any of the sites.

Plants may show a strong response to N-enriched soil patches even when P is limiting. Provided there is demand for N, Robinson (1996) suggested that roots do not necessarily respond any less to N than they do to P. NUE of N at sites A and C was high compared to other floodplain forests in southeastern United States (Watt and Golladay 1999, Lockaby and Conner 1999), suggesting that N was strongly retained prior to senescence and demand for N was potentially high. The response to microsite N

fertilization in my study may also have been a result of strong competition for N among different forest species. Although root proliferation does not necessarily increase N capture for plants grown in isolation (Jackson and Caldwell 1996), proliferation may increase N capture when plants compete (Robinson 2001, Hodge et al. 1999).

The mode of root proliferation within N enriched microsites was slightly different between sites A and C. At site A, the increase in root length density was caused largely by an increase in root biomass. However, neither root biomass nor specific root length responded significantly to the fertilization treatments at site C though there were trends for an increase in both. Thus, the increase in root length density was caused by some combination of a decrease in root diameter and an increase in root biomass. These data suggest that use of root length density as a measure of root proliferation may be more sensitive than root biomass alone, especially when changes in root biomass and diameter are acting concurrently.

The lack of a significant response to microsite N fertilization at site B was somewhat puzzling. Total soil N, mineralizable N in soil, and N:P ratio was lowest and N NUE was highest at this site when compared to sites A and C. Thus, root proliferation within N enriched microsites should have been greatest at this site because of the relatively high demand for N. There are two likely possibilities to explain why roots did not respond to N at site B. First, subtle differences in forest vegetation may explain the lack of root response to N at site B. *Pinus taeda* was a dominant overstory species at only this site and may be a weak root proliferator compared to hardwood species. Mou et al. (1997) and Einsmann et al. (1999) found that *P. taeda* exhibited a weaker root proliferation response to nutrient-rich patches than *L. styraciflua* when plants were grown alone in pots. However, it is not clear whether the large response shown by *L. styraciflua* is common to other floodplain hardwood species. A study by van Vuuren (2003) showed that *A. rubrum* responded to organic nutrient patches via root proliferation, although the response was much smaller than in *Betula populifolia* Marsh. Second, calcium (Ca) was much lower at this site than the other two sites and also lower than most soils (Troeh and Thompson 1993). Therefore, Ca may have been limiting instead of N or P.

It was also surprising that there was no response to microsite P enrichment at any of the sites, considering the finding that N:P ratios indicated P limitation. Jackson and

Caldwell (1989) found that *Artemesia tridenta* (Rydb.) Beetle proliferated roots in N and N-P-K patches but not P patches, which they attributed to high demand for N and low demand for P. Similarly, Mou et al. (1995) showed strong correlations between fine root biomass and soil P and K but not N in a southeastern pine forest. In my study, however, demand for P was most likely high because P NUE was greater relative to other floodplain forests in southeastern United States (Watt and Golladay 1999, Lockaby and Conner 1999). Although there may be many reasons why roots did not respond to P, I think four are likely.

First, P may have become rapidly unavailable to plants due to adsorption to clay particles. P may have been quickly sorped into clay particles before plant uptake could take place. Craft and Casey (2000) suggested that P accumulation in floodplain wetlands at the Jones Ecological Research Center in Newton, Georgia, was at least partly due to P sorption. P sorption is especially high when soil pH<5, and pH of soils at my study sites were close to 5.

Second, plants may foraging for P by changing root system physiology rather than proliferating roots into P-enriched microsites. P-patches were relatively transient because TSP was released within 2 weeks after application, whereas N patches were more permanent because coated urea was released slowly over the course of the study. Relatively faster types of plasticity, such as physiological plasticity, may be more important in ephemeral patches (Jackson and Caldwell 1996, Robinson 1996). However, Robinson (2001) predicted that roots should respond using physiological plasticity immediately and switch to morphological plasticity as the patch becomes depleted. Fine roots may also have proliferated initially but died once the patch became completely depleted.

Third, floodplain forest species may possess genes for root proliferation in response to N-rich patches but not for P-rich patches. Co-occuring species have been found to show different degrees of proliferation response to P-rich patches. Blair and Perfecto (2004) found that 2 of 7 tropical tree species did not respond to P-rich patches, even though one of these species, *Cordia alliodora* (Ruiz & Pav.) Oken, responded to patches enriched with nitrogen, phosphorus, and potassium (Huante et al. 1998). Root proliferation for NO_3 in N deficient plants is at least partly under genetic control (Zhang

and Forde 1998, 1999, and 2000), and it is likely that there are genes that control for root proliferation in P-rich patches. In southeastern United States, floodplain forest productivity is typically limited by N (Lockaby and Conner 1999). Therefore, species within these forests may be adapted to N-limited conditions and respond only to N-rich patches via proliferation.

Fourth, N:P ratios may not be a good indicator of what nutrient is limiting at my sites. Another nutrient (e.g. Ca, Mg) may be limiting, but I think this is unlikely because both N NUE and P NUE are high at all of my sites. Alternatively, N may limit plant growth even though N:P ratios suggest P limitation. N limitation or co-limitation has been observed at high N:P ratios (Venterink et al. 2003). Furthermore, Güsewell and Koerselmann (2002) found that N limitation can occur at high litter N content. Therefore, it is possible that plants did respond to the limiting nutrient in my study, which was nitrogen.

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Table 4.1 Soil properties of three floodplain sites along Chickasawatchee Creek. Total C and N were measured on a CHN autoanalyzer. Available N was determined using an anaerobic incubation. P, K, Ca, Mg, and Fe were measured using the Mehlich III technique.

Site	pH	Total C (%)	Total N (%)	Available N (µg/g)	P (µg/g)	K (µg/g)	Ca (µg/g)	Mg (µg/g)	Fe (µg/g)
A	5.2	1.8	0.08	26	2	12	890	12	49
B	4.2	1.7	0.06	17	2	14	90	11	55
C	5.3	1.3	0.07	75	3	12	570	13	51

Table 4.2 Litter nutrient content, N:P ratios, and nutrient use efficiency (NUE) of the three floodplain sites along Chickasawatchee Creek. NUE is the dry mass to nutrient content ratio.

Site	N content (mg/g)	P content (mg/g)	N:P	N NUE	P NUE
A	8.2	0.3	24	124	2990
B	8.0	0.4	18	131	2330
C	11.9	0.5	21	93	1950

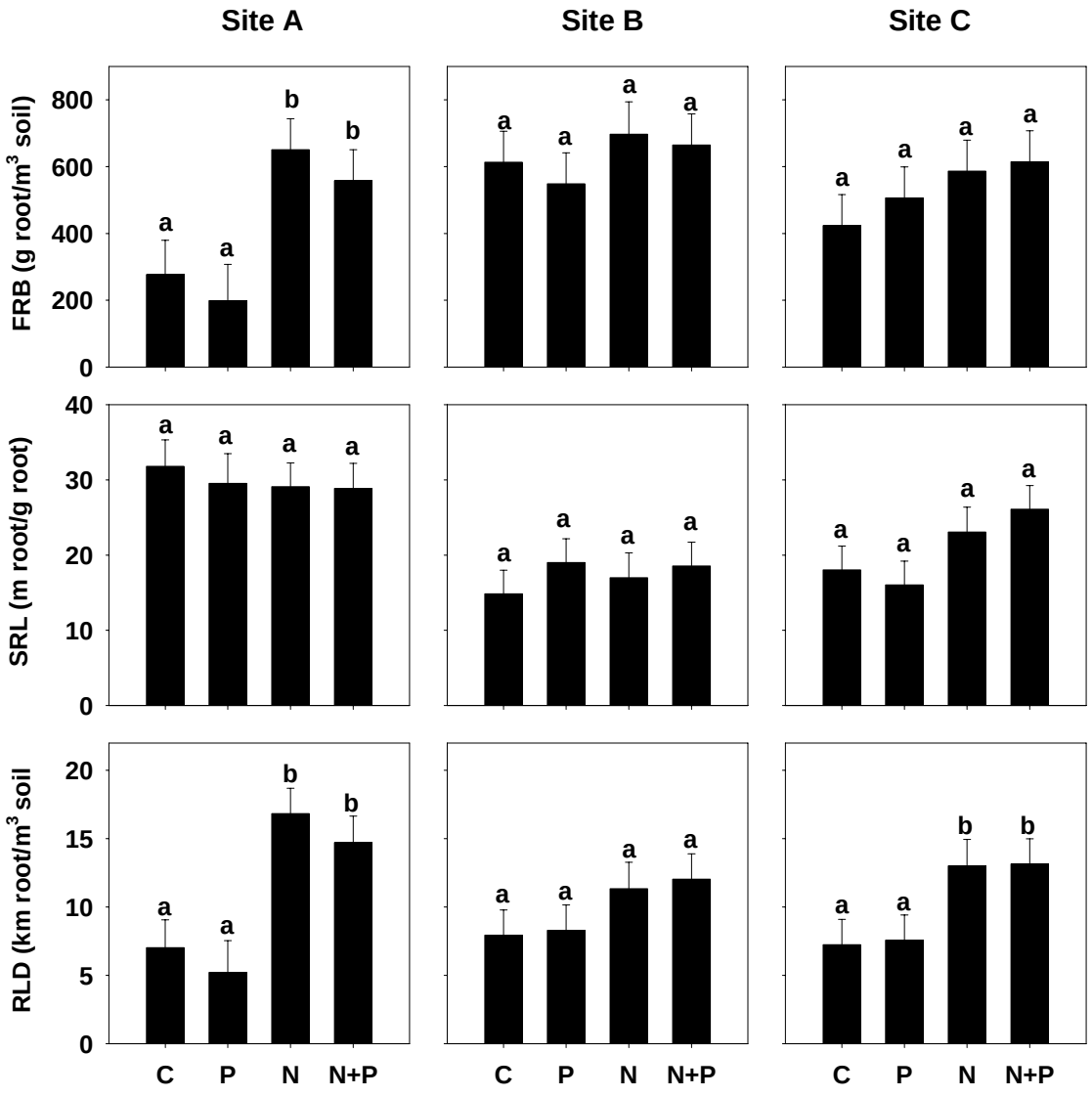

Figure 4.1 Fine root response to microsite fertilization (+1 SE). C = control, P = phosphorus fertilization only with triplesuperphosphate (100 kg P/ha) N = nitrogen fertilization only with coated urea (400 kg N/ha), N + P = nitrogen and phosphorus fertilization. FRB = fine root biomass, SRL = specific root length, and RLD = root length density. Different letters indicate significant differences among treatments (p<0.05).

Chapter 5. Influence of oxygen heterogeneity on nutrient foraging in three bottomland hardwood species

Abstract

I hypothesized that low soil oxygen would suppress the well-known root proliferation response of plants to nutrient patches and that the suppression would be greater for flood intolerant than for flood tolerant species. To test these hypotheses, seedlings of three bottomland hardwood species that vary in flood tolerance (*Nyssa aquatica* > *Fraxinus pennsylvanica* > *Liquidambar styraciflua*) were grown in pots exposed to various combinations of nutrient and waterlogging heterogeneity. All species were precise foragers (preferential proliferation of roots in nutrient-rich patches) under aerobic conditions. In the least flood tolerant species, *L. styraciflua*, waterlogging suppressed this response and resulted in reduced total shoot and root biomass. In contrast, biomass of both *F. pennsylvanica* and *N. aquatica* were both greater when nutrients were heterogeneous compared to homogeneous and when waterlogged compared to drained. Nutrients may have been easier to acquire under waterlogged conditions due to faster diffusion rates. These results indicate that the combination of soil anoxia and tolerance of flooding may influence not only total biomass growth but also root system behavior across a flooding gradient.

Introduction

Tree seedling establishment is critically dependent on nutrient acquisition within soils. The ability to obtain nutrients is influenced by the effects of many environmental factors and various interactions with other plants, including drought, competition for light, and herbivory. It is also well recognized that soils are heterogeneous at scales relevant to individual plants (Lechowicz and Bell 1991, Jackson and Caldwell 1993a, 1993b, Ehrenfeld et al. 1997), and many plants respond to this heterogeneity through altering their root system morphology via proliferating roots into nutrient-rich patches (Robinson 1994, Hodge 2004). This type of behavior in plants has often been referred to as "precision" (e.g. Campbell et al. 1991, Einsmann et al. 1999, Robinson et al. 1999). Some plants are also "sensitive" to nutrient heterogeneity because growth is greater when nutrients are spatially heterogeneous than when the same amount of nutrient is homogeneous (e.g. Birch and Hutchings 1994, Wijesinghe and Hutchings 1997, Einsmann et al. 1999, Day et al. 2003, Blair and Perfecto 2004).

Precise foraging increases nutrient capture, especially when plants are competing for nutrients with other plants (Hodge et al. 1999, Robinson 2001). Therefore, root proliferation is often advantageous to plants, but certain conditions may limit benefits. Root proliferation in nutrient-rich patches can be reduced by shading (Huante et al. 1998a,b), competition with microbes (Hodge 2004) or other plants (Bliss et al. 2003), high fertility (Friend et al. 1990), or associations with mycorrhizal fungi (Tibbett 2000).

In bottomland hardwood forests of southeastern United States, soil anoxia may stress root systems and reduce root proliferation. Root production of bottomland hardwood species is often lower in anoxic soils relative to well aerated conditions (e.g. McLeod et al. 1986, Pezeshki 1991, Megonigal and Day 1992, Clawson et al. 2001, Burke and Chambers 2003). Furthermore, plants that are flood intolerant typically lose the ability to take up and retain nutrients under anoxic conditions (McKevlin et al. 1998). However, many bottomland hardwood species have adaptations, such as the formation of adventitious roots or aerenchyma to promote root aeration (Blom and Voesenek 1996, McKevlin et al. 1998), that may compensate for the stresses of anoxia. These adaptations may allow species to precisely forage for nutrients in anoxic soils.

Bottomland hardwood forests are typically flooded for part of the growing season, and spatial heterogeneity in soil anoxia is likely to change with the annual hydroperiod. For example, soils may be uniformly anoxic in early spring and become aerobic when soils are no longer saturated. As soils dry during spring, oxygen-rich and oxygen-poor patches may coincide within the rooting zone of individual plants. The availability of mineral nutrients may also change with the annual hydroperiod. Soil anoxia may facilitate the loss of nitrogen via denitrification or shift the mineral form to ammonium due to a lack of nitrification (Blom and Voesenek 1996). In contrast, phosphorus availability may increase under anoxic conditions because the reduction of iron (Fe) will release soluble phosphorus into the soil (Bridgham et al. 1998). Slower decomposition under anoxic conditions also may shift both P and N to organic forms that are not available to plants (Craft 1997, Craft and Chiang 2002). Therefore, roots of bottomland hardwood species may need to deal concurrently with heterogeneously distributed soil oxygen and nutrients, which ultimately may affect a species' growth, survival, and production.

Here, I investigated how the combination of soil oxygen and nutrient heterogeneity affected root foraging for nutrients and biomass of three bottomland hardwood species: *Liquidambar styraciflua* L., *Fraxinus pennsylvanica* Marsh., and *Nyssa aquatica* L. I used a pot study where the spatial distribution of soil anoxia and mineral nutrients was manipulated. The three species co-occur in bottomland hardwood forests but differ in their tolerance to soil anoxia: *N. aquatica* > *F. pennsylvanica* > *L. styraciflua* (Hook 1984). I addressed the following questions and related hypotheses:

1) Do seedlings precisely locate roots within nutrient-rich areas under aerobic conditions? I predicted that all species would be precise foragers.

2) Does waterlogging affect root proliferation? I expected that avoidance of waterlogging would overwhelm the root proliferation response to patchy nutrients in *L. styraciflua*. In contrast, waterlogging would not affect foraging in *N. aquatica*. Response of *F. pennsylvanica* would be intermediate.

3) Does waterlogging and/or nutrient heterogeneity affect seedling biomass growth? I predicted that biomass growth would be positively related (i.e. sensitive) to nutrient heterogeneity. However, biomass production of *L. styraciflua* would be negatively affected by waterlogging, whereas *N. aquatica* would be unaffected by waterlogging. Biomass response of *F. pennsylvanica* would be intermediate.

Methods

Experimental design

This experiment was conducted using potted plants in the Virginia Tech glasshouses (Blacksburg, Virginia, USA) during the 2003 growing season. *L. styraciflua* and *F. pennsylvanica* seeds were obtained from Sheffield's Seed Company (Locke, New York). *L. styraciflua* and *F. pennsylvanica* seed sources were California and North Dakota, respectively. *N. aquatica* seeds were collected in November 2002 from a bottomland hardwood forest in the Chickasawatchee Wildlife Management Area, Calhoun County, Georgia. In April 2003, seeds of each species were germinated in vermiculite on a mist bench with a heat source. After the emergence of cotyledons, seedlings were transplanted to a 5.1 diameter × 5.1-cm long section of PVC pipe filled with perlite. I clipped the end of the taproot to promote development of lateral roots. Seedlings received a small amount of fertilizer immediately after transplantation.

On 17 June 2003, seedlings of similar size were transplanted individually into two 10.7-cm^3 square pots connected on one side (Figure 5.1). Hereafter, the two-pot system will be referred to as "the pot" consisting of two separate sides. I created waterlogged conditions in one side of the pot and drained conditions in the other side. Pots were filled with 20:1 mixture of construction grade sand to floodplain soil that was collected from the Chickasawatchee Wildlife Management Area. Construction grade sand is naturally low in soil nutrient content. I added a small amount of floodplain soil to inoculate the sand with native microbes. All pots were painted white to reduce heating the sand.

Each pot was drained, waterlogged, or partially waterlogged. Drained pots had drainage holes at the bottom of the pot, whereas waterlogged pots had drainage holes

near the soil surface. Partially waterlogged pots, had drainage holes near the soil surface on one side of the pot and holes at the bottom on the other side (Figure 5.1). Within each pot, 1.0 g of slow-release, Osmocote fertilizer (15-9-12 NPK, 7% ammoniacal-N, 8% nitrate-N; The Scotts Company, Marysville, Ohio, USA) was either evenly distributed between both sides of the pot (homogeneous) or concentrated in one side of the pot (heterogeneous). Therefore, each pot received the same total amount of fertilizer, which was intended to release the equivalent of 70 kg/ha of N, a typical value for a bottomland hardwood forest (Burke et al. 1999). The fertilizer was mixed into the top 3-cm of sand in each pot.

I created a total of seven nutrient/waterlogging treatments (Table 5.1). In two of the seven treatments, both sides of the pot were drained, and fertilizer was either homogeneously (HOM-D) or heterogeneously (HET-D) distributed. In two other treatments, both sides of the pot were waterlogged, and fertilizer was either homogeneous (HOM-W) or heterogeneous (HET-W). The final three treatments were partially waterlogged but differed in that fertilizer was either evenly distributed over both sides of the pot (HOM-P), concentrated in waterlogged (HET-P_w) side of the pot, or concentrated in drained side (HET-P_d) of the pot.

Prior to seedling transplantation into experimental pots, a plastic ring with a slightly greater diameter than the PVC sections containing the seedlings were placed in the middle of each pot, bisecting the divider of the two sides. The ring was filled with small Styrofoam balls. The PVC section along with its seedling were placed into the ring. The Styrofoam balls prevented wicking of water from waterlogged to drained halves but did not impede root growth from the PVC sections to the experimental pots. Seedlings were watered two times a day for five minutes by an overhead irrigation system to ensure that all pots received the same amount of water. I used this irrigation interval to maintain drained pot halves near field capacity and to minimize nutrient leaching.

Harvest

In late September 2003, plants were harvested over a two-week period. Aboveground material was clipped to the soil surface. Roots were separately washed for

each side of the pot over a 1-mm sieve to remove sand and sorted to fine roots (< 2 mm) or coarse roots (> 2 mm). I used a subsample of fine roots to estimate specific root length (SRL = cm per g of root). The subsample was scanned, and root length was determined using WinRhizo software (Regent Instruments, Quebec, Canada). All plant parts were dried to a constant weight at 60˚C and weighed. SRL of each root subsample from each side of a pot was multiplied by its corresponding fine root biomass to yield total fine root length.

Waterlogging and nutrient test

I monitored the effectiveness of fertilization and waterlogging in six extra pots without plants for each treatment. Two steel welding rods were placed in each pot at the beginning of the experiment. At same time when plants were harvested in other pots, welding rods were removed and the depth of rust on the rod was recorded as an indicator of well-oxygenated conditions (Bridgham et al. 1991). I ran a 2.0 M KCl extraction to on a subsample of sand from each pot half. Nitrogen (NH_4-N and NO_3-N) content in each extract was measured on an autoanalyzer (Lachat Quickchem AE autoanalyzer, Lachat Instruments, Milwaukee, Wisconsin).

Data analysis

Rust depth on steel welding rods was compared between waterlogged and drained pot sides using a one-way ANOVA. Although this analysis violates the assumption of independent samples, I used it as a diagnostic tool. I determined the effectiveness of fertilization among treatments using a MANOVA with NH_4-N and NO_3-N per pot as dependent variables. I also used MANOVA to compare the N difference (NH_4-N or NO_3-N in one side of the pot minus NH_4-N or NO_3-N in the other side of the pot) among treatments.

To measure root foraging, I calculated a root foraging index (RFI) by subtracting fine root length in nutrient-rich side of the pot minus the nutrient-poor side (heterogeneous fertilizer) or left minus right side (homogeneous fertilizer). In HOM-P pots, I subtracted root length in the waterlogged side by the drained side. For all treatments, the difference in root length was divided by the total fine root length per pot

(*en sensu* Mou et al. 1997) to correct for differences in plant size. To test for differences in precision, I ran a two-way ANOVA with species and treatment as main effects and RFI as the response variable using the GLM procedure of SAS version 9 (SAS institute, Cary, North Carolina). I used 95% confidence intervals about least square means and checked the intervals for overlap of zero. Seedlings were precise foragers if RFI was greater than zero when nutrients were distributed heterogeneously and not different from zero when nutrients were homogeneous. In HOM-P and HET-P_w pots, seedlings avoided waterlogged conditions if RFI was less than zero despite the presence of fertilizer.

Also, I used a series of *a priori* orthogonal contrasts within the ANOVA model to address my specific root foraging questions for each species. To determine whether seedlings were precise foragers in aerobic conditions, I contrasted RFI between HET-D and HOM-D pots. Species exhibited precision if RFI was greater in HET-D pots. To ascertain the effects of waterlogging on foraging, I compared HET-D to HET-W pots and HET-P_w to HET-P_d pots. Waterlogging depressed root proliferation if RFI was lower in HET-W pots relative to the HET-D pots and shifted proliferation if RFI was different between HET-P_w and HET-P_d pots.

The effects of waterlogging and/or nutrient heterogeneity on shoot and root biomass were examined using MANOVA with species and treatments as main effects. I performed univariate ANOVAs with the same contrasts as for precision, except I compared (HOM-D & HET-D) vs. (HOM-W & HET-W) to determine whether waterlogging increased or decreased biomass.

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Results

Treatment effects on soils

Rust depth was >4× greater in drained than in waterlogged pot halves ($F_{1,183}$ = 1400, $p < 0.0001$, data not shown). N (NH_4-N and NO_3-N) per pot was not different among the seven treatments (Wilks' lambda, $F_{12,68} = 1.3$, $p = 0.26$), but N difference between the two halves of the pots was 3-5× greater in treatments where fertilizer was heterogeneous (HET-D, HET-W, HET-P_d, and HET-P_w) than when homogeneous (HOM-D, HOM-W, and HOM-P) ($F_{2,34}$ =14.9, $p < 0.0001$).

Root foraging

The combination of nutrient and waterlogging heterogeneity had different effects on the foraging behavior and biomass of the three species (Figure 5.2). I detected significant treatment ($F_{6,251} = 8.8$, $p < 0.0001$) and interaction ($F_{12,251} = 5.6$, $p < 0.0001$) effects on RFI. For all species, RFI was not significantly different from zero in pots where nutrients were distributed homogeneously, suggesting that root systems developed symmetrically (Figure 5.2). RFI was significantly greater in HET-D than in HOM-D pots for *L. styraciflua* and *N. aquatica* (Figure 5.2, Table 5.2), indicating that these species were precise foragers. *F. pennsylvanica* seemed to exhibit a trend toward precision because RFI was significantly greater than zero in HET-D pots. The proliferation response was not depressed in any of the species when both halves of the pot were waterlogged (Figure 5.2, Table 5.2). RFI was not different between HET-D and HET-W pots. When waterlogging was heterogeneous, root foraging for nutrients of *L. styraciflua* was strongly affected (Figure 5.2, Table 5.2). Roots of this species tended to avoid waterlogging even when the fertilizer was concentrated on the waterlogged side of the pot. RFI in HOM-P and HET-P_w pots was significantly negative and different between HET-P_d and HET-P_w pots. In contrast, waterlogging heterogeneity did not seem to affect foraging for nutrients of *F. pennsylvanica* (Figure 5.2, Table 5.2). RFI of HET-P_d and HET-P_w pots were not different and both were greater than zero. Although RFI of *N. aquatica* was not different between HET-P_d and HET-P_w pots, there was a slight preference for waterlogging because RFI was significantly positive in HET-P_w, but not in HET-P_d pots.

Seedling biomass

There were significant treatment (Wilks' lambda, $F_{12,460} = 2.7$, $p < 0.01$), species ($F_{4,460} = 109.3$, $p < 0.0001$), and interaction ($F_{24,460} = 3.8$, $p < 0.0001$) effects on biomass (Figure 5.3). Both shoot and root biomass of *F. pennsylvanica* and shoot biomass of *N. aquatica* were greater in HET-D compared to HOM-D pots, suggesting that these species were sensitive to nutrient heterogeneity (Table 5.2, Figure 5.3). Waterlogging reduced both shoot and root biomass of *L. styraciflua*. In contrast, shoot biomass of *F. pennsylvanica* and root and shoot biomass of *N. aquatica* were increased under

waterlogging. Each species was affected when waterlogging was heterogeneous, but in different ways. Shoot and root biomass of *L. styraciflua* were lower in HET-P_w pots relative to HET-P_d pots, whereas shoot and root biomass of *F. pennsylvanica* were greater in HET-P_w pots. Shoot biomass of *N. aquatica* was greater in HET-P_w relative to HET-P_d pots but not for root biomass.

Discussion

Do seedlings precisely locate roots within nutrient-rich areas under aerobic conditions?

My prediction that all species would precisely forage for nutrient-rich patches under aerobic conditions was generally supported. *L. styraciflua* showed the highest precision, followed by *N. aquatica* and *F. pennsylvanica*. My finding of precise foraging for *L. styraciflua* when grown in isolation is consistent with other studies (Ludovici and Morris 1996, Mou et al. 1997, Einsmann et al. 1999). However, Bliss et al. (2002) found that precision of *L. styraciflua* was reduced when in multi-plant monocultures. They suggested that antagonisms between root systems may have reduced precision. Many other tree species have been shown to precisely forage for nutrients. Empirical studies have shown precise foraging in conifers (e.g. Coutts and Philipson 1976, Friend et al. 1990, George et al. 1997, Mou et al. 1997), temperate hardwoods (Ludovici and Morris 1996, Mou et al. 1997, Einsmann et al. 1999, van Vuuren et al. 2003), and tropical species (Huante et al. 1998a, 1998b, Blair and Perfecto 2004).

Does waterlogging affect root proliferation?

I predicted that waterlogging would depress and overwhelm root foraging for nutrients in *L. styraciflua*, not affect foraging in *N. aquatica*, and have an intermediate effect on *F. pennsylvanica.*. Again, my results largely support my predictions. Waterlogging did not depress the magnitude of precision exhibited by *L. styraciflua*. However, *L. styraciflua* roots tended to avoid waterlogging, preferentially placing roots in drained areas that were nutrient-poor and avoiding waterlogged areas that were nutrient-rich. Although I expected that *L. styraciflua* would tend to avoid waterlogging, I was surprised by the degree of avoidance especially since *L. styraciflua* seedlings have been shown to survive continuous flooding of more than two years (Angelov et al. 1996).

Foraging of *F. pennsylvanica* and *N. aquatica* were not affected by waterlogging, and *N. aquatica* showed a slight preference for waterlogging. My findings with *F. pennsylvanica* and *N. aquatica* are consistent with the relative high flood tolerance of these species (Pezeshki and Chambers 1986, McLeod et al. 1986).

Does waterlogging and/or nutrient heterogeneity affect seedling biomass growth?

I predicted that biomass of all three species would be positively related to nutrient heterogeneity because nutrient acquisition may be more efficient under heterogeneous than homogeneous conditions (Jackson and Caldwell 1996). In my study, *F. pennsylvanica* and *N. aquatica* were sensitive as expected but not *L. styraciflua*. Other studies have reported conflicting results for sensitivity among tree species. Blair and Perfecto (2004) showed increased aboveground biomass under heterogeneous conditions in 2 of 3 tropical species. However, van Vuuren (2003) found that biomass of *Acer rubrum* and *Betula populifolia* was not different when nutrients were supplied heterogeneously or homogeneously, which they suggested was due to very little contrast in the amount of nutrients between rich and poor areas. Similarly, Mou et al. (1997) observed no sensitivity in *L. styraciflua* and *Pinus taeda*, but they attributed the lack of sensitivity to high overall level of nutrient availability in homogeneous and heterogeneous conditions. In contrast, Einsmann et. al (1999) found that *L. styraciflua* was sensitive even though *P. taeda* was not. Bliss et al. (2003) did not observe any increase in biomass under heterogeneous conditions for either *L. styraciflua* or *P. taeda* when species were grown in monoculture or with five other co-occurring species. However, *P. taeda* was sensitive when grown in competition with an annual herb, *Erichtites hieracifolia*, suggesting that sensitivity is context specific.

Biomass of *L. styraciflua* was strongly affected by the overall level of waterlogging. Not surprisingly, waterlogging reduced both shoot and root biomass. *L. styraciflua* seedlings were also influenced by the spatial distribution of waterlogging and nutrients. Shoot biomass was lower when nutrient-rich patches and waterlogging coincided compared to when they did not coincide. The effects of waterlogging on *L. styraciflua* in my study are similar to those reported by Angelov et al. (1996). They found that *L. styraciflua* seedlings that were continuously flooded survived for more than two years, but height and diameter of shoots were both 25% less than in non-flooded

seedlings. In experimental tanks, Battaglia et al. (2000) also showed that mortality was greater and height growth lower for *L. styraciflua* seedlings in elevated mounds compared to saturated pits.

Waterlogging tended to increase biomass of *N. aquatica* and *F. pennsylvanica* seedlings, and both performed well when waterlogging and nutrient-patches coincided. Other studies, however, have reported conflicting responses to soil anoxia for these two species. Pezeshki and Chambers (1986) found that flooding did not cause a reduction in photosynthesis of *F. pennsylvanica* seedlings. Similarly, McLeod et al. (1986) showed flooding at ambient temperatures did not significantly decrease biomass of *N. aquatica* seedlings. However, Gravatt and Kirby (1998) reported a 25% and 30% reduction in *N. aquatica* and *F. pennsylvanica* seedlings respectively compared to non-flooded controls. It is surprising that biomass of these two species was higher under waterlogged conditions. There are two possibilities that may explain this response. First, water may have limited biomass growth in drained pots. I think this is unlikely because drained pots were well watered throughout the experiment. Second, nutrients may have been easier to acquire under waterlogged conditions. In pots without plants, nitrogen availability was not different among treatments, suggesting that leaching in drained and waterlogged pots was similar. However, the rate at which nutrients arrived at the root surface may have been augmented by waterlogging. Both mass flow and diffusion of mineral nutrients decrease when larger soil pores are no longer filled with water because pathways to roots become more tortuous (Bowen 1984). Therefore, *N. aquatica* and *F. pennsylvanica* seedlings may have benefited from a faster flow of nutrients from sand to root surface in waterlogged relative to drained pots.

My data provide strong evidence that the effects of waterlogging and nutrient heterogeneity on root foraging behavior and biomass may be related to flood tolerance. *L. styraciflua* was a precise forager under aerobic conditions, but waterlogging overrode foraging and reduced biomass. In contrast, waterlogging had little effect on foraging of *N. aquatica* and *F. pennsylvanica* and tended to increase biomass in both species. My study shows that the spatial distribution of nutrients and waterlogging can have strong effects on foraging for nutrients and biomass growth, which ultimately may affect

seedling growth and survival during early stages of establishment in bottomland hardwood forests.

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Table 5.1 Description of the seven treatments used in nutrient/waterlogging experiment. One gram of fertilizer was applied to each pot (15-9-12 NPK).

Treatment	Description
HOM-D	Fertilizer broadcast evenly over both sides of the pot; both sides of the pot drained
HET-D	Fertilizer concentrated in one side of the pot; both sides of the pot drained
HOM-W	Fertilizer broadcast evenly over both sides of the pot; both sides of the pot waterlogged
HET-W	Fertilizer concentrated in one side of the pot; both sides of the pot waterlogged
HOM-P	Fertilizer broadcast evenly over both sides of the pot; one side of the pot waterlogged, one side drained
HET-P_w	Fertilizer concentrated in waterlogged side of the pot; one side of the pot waterlogged, one side drained
HET-P_d	Fertilizer concentrated in drained side of the pot; one side of the pot waterlogged, one side drained

Table 5.2 Significant orthogonal contrasts for RFI, shoot biomass, and root biomass of the three species (*Liquidambar styraciflua*, *Fraxinus pennsylvanica*, *Nyssa aquatica*). Treatments are described in Table 1. Each contrast has one degree of freedom. RFI = root foraging index.

Variable	Species	Contrast	F-value	*p*
RFI	F. pennsylvanica	HOM-D vs. HET-D	12.1	0.0006
	N. aquatica	HOM-D vs. HET-D	8.6	0.0037
	L. styraciflua	HET-P_d vs. HET-P_w	43.2	<0.0001
Shoot biomass	F. pennsylvanica	HOM-D vs. HET-D	4.6	0.0338
	N. aquatica	HOM-D vs. HET-D	4.6	0.0331
	L. styraciflua	(HOM-D & HET-D) vs. (HOM-W & HET-W)	21.7	<0.0001
	F. pennsylvanica	(HOM-D & HET-D) vs. (HOM-W & HET-W)	5.9	0.0163
	N. aquatica	(HOM-D & HET-D) vs. (HOM-W & HET-W)	13.7	0.0003
	L. styraciflua	HET-P_d vs. HET-P_w	4.2	0.0414
	F. pennsylvanica	HET-P_d vs. HET-P_w	14.9	0.0001
	N. aquatica	HET-P_d vs. HET-P_w	5.9	0.0156
Root biomass	F. pennsylvanica	HOM-D vs. HET-D	11.0	0.0010
	L. styraciflua	(HOM-D & HET-D) vs. (HOM-W & HET-W)	18.0	<0.0001
	N. aquatica	(HOM-D & HET-D) vs. (HOM-W & HET-W)	8.0	0.0051
	F. pennsylvanica	HET-P_d vs. HET-P_w	35.0	<0.0001

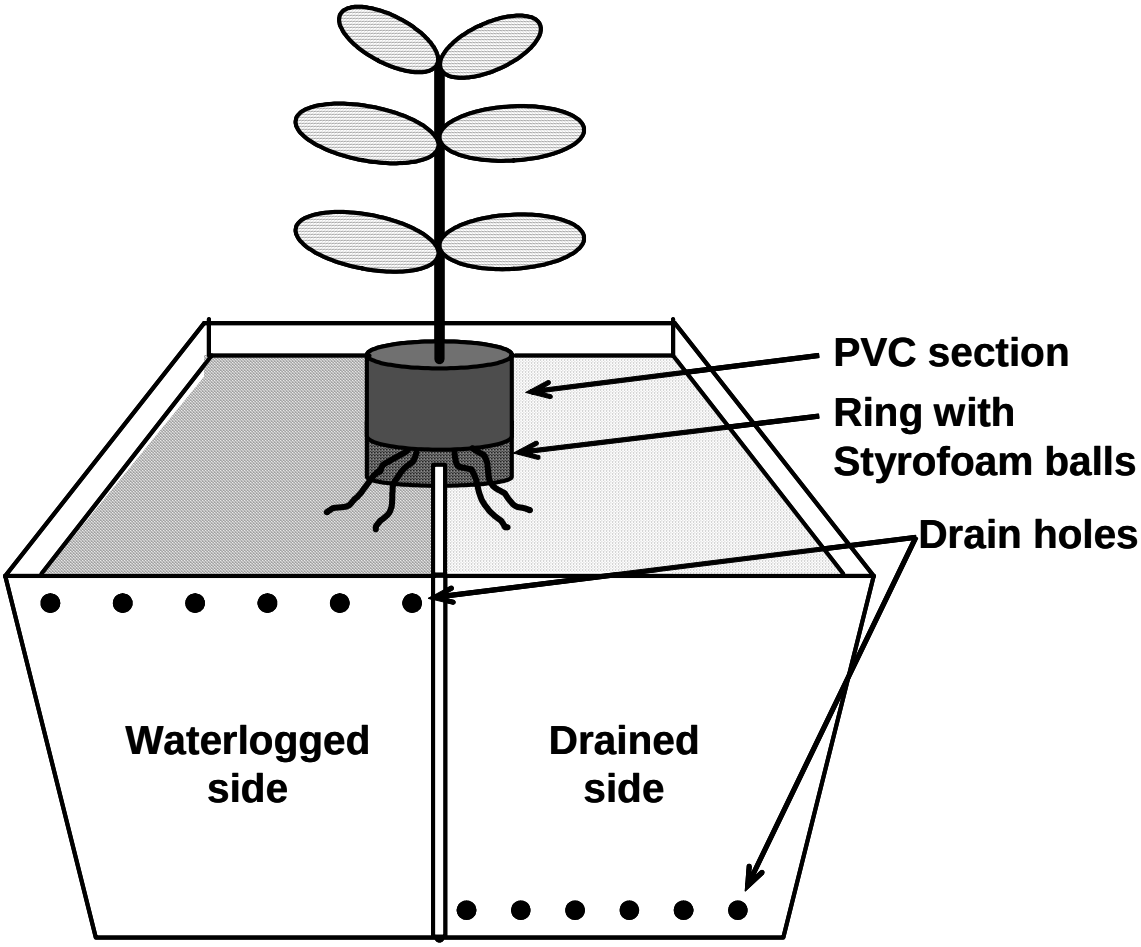

Figure 5.1 Example of tree seedling in two-pot system. The pot pictured is the DW treatment where one side of the pot is waterlogged and the other side is drained. Tree seedlings where placed in the middle of the two pots on ring containing Styrofoam balls that prevented movement of water from one side of the pot to the other but did not impede root growth.

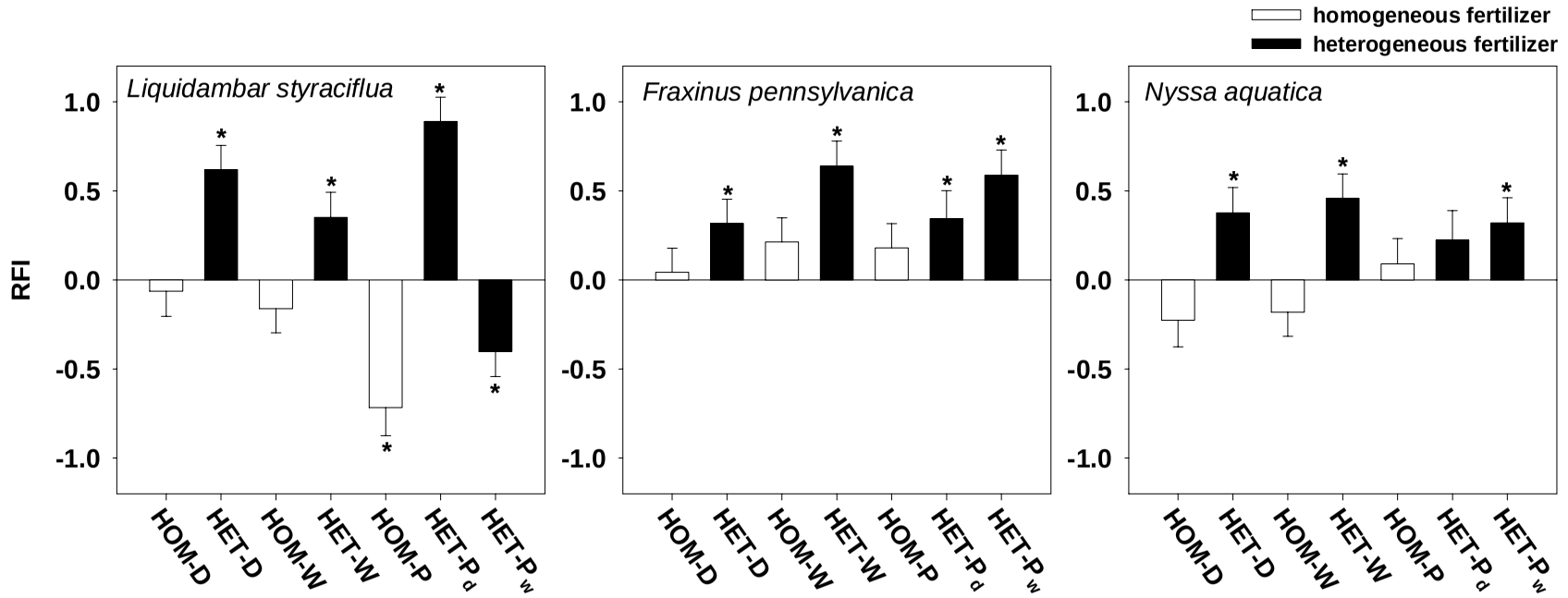

Figure 5.2 Precision (measured by RFI) of the three tree species (*Liquidambar styraciflua*, *Fraxinus pennsylvanica*, and *Nyssa aquatica*). Treatments are describe in Table 5.1. RFI is calculated as the difference in fine root length between the two sides of the pot divided by total root length per pot. For HET-D, HET-W, HET-P_d, and HET-P_w pots, this is the nutrient-rich minus the nutrient-poor side. For HOM-D and HOM-W pots, this is the left side minus the right side. For HOM-P pots, this is the waterlogged side minus the drained side. An asterisk (*) indicates when RFI is significantly different than zero. Bars are least squares means +1 SEM.

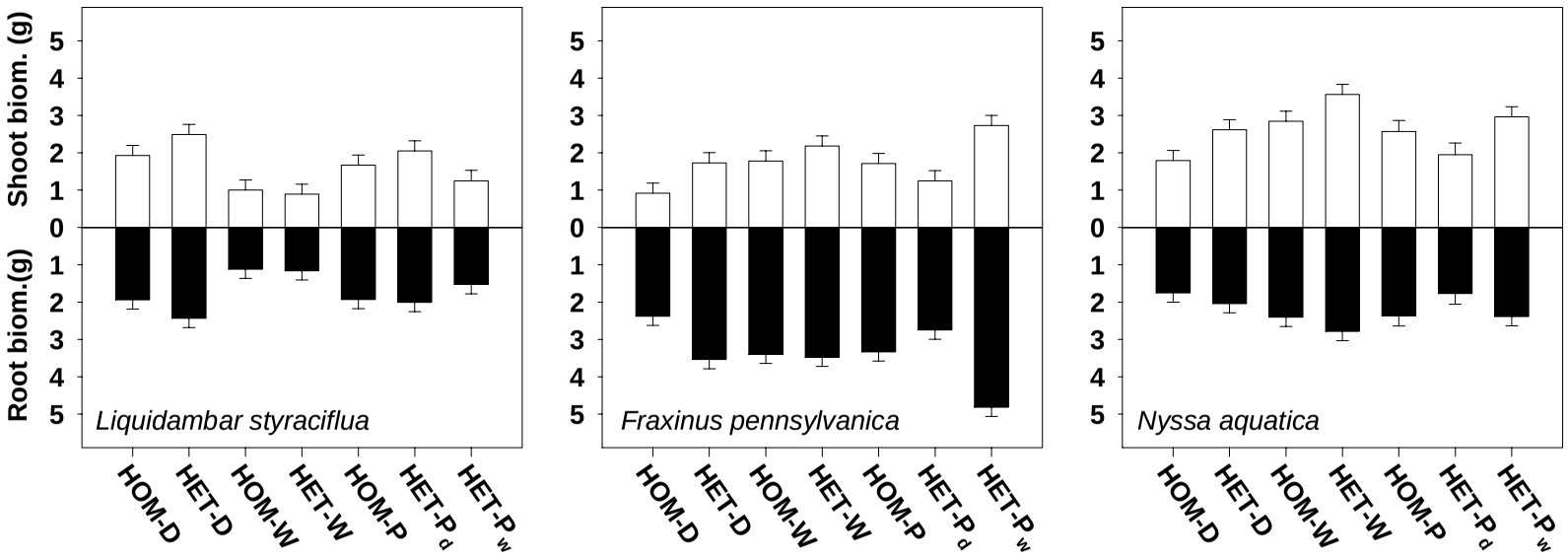

Figure 5.3 Root and shoot biomass of the three species (*Liquidambar styraciflua*, *Fraxinus pennsylvanica*, and *Nyssa aquatica*) in the seven treatments. Treatments are described in Table 5.1. White and black bars are shoot and root biomass, respectively. Bars are least squares means +1 SEM.

Summary

The previous chapters examined how nutrient and/or oxygen availability influenced root proliferation into nutrient-rich patches in forested wetland ecosystems. Several key findings emerged for my data:

1. In natural soil, root biomass was not correlated with soil nutrient availability at local scales but was strongly correlated with nutrient availability at the ecosystem scale (Chapter 2).
2. In sand ingrowth cores, root response to phosphorus heterogeneity did not differ between nutrient-rich (floodplain) and nutrient-poor (depressional) swamps. In addition, roots generally did not respond positively to phosphorus-rich patches (Chapter 3). However, problems with the growth medium (sand) made the results inconclusive.
3. In native soil ingrowth cores, roots generally responded to nitrogen-rich patches but not to phosphorus-rich patches even though litter N:P ratios suggested that phosphorus might limit plant growth in these ecosystems (Chapter 4).
4. The lack of response to phosphorus may be a general characteristic of plants in these ecosystems (Chapters 3 and 4).
5. The combination of oxygen and nutrient heterogeneity strongly affected root proliferation into nutrient-rich patches and biomass growth, which appeared to be related to flood tolerance (Chapter 5).

Overall, my data show and further suggest that environmental factors, particularly soil oxygen availability, may affect root foraging for nutrients in forested wetland ecosystems. Therefore, the environmental context where plants reside may determine the degree of proliferation. Most studies to date have failed to examine environmental factors that may limit root proliferation. My results show that ecologists should be keenly aware of the environmental context that may either positively or negatively affect root proliferation when examining the importance of this phenomenon.

My study also demonstrates the need to explore root proliferation under field conditions. Most studies examining root proliferation have been done using individual

plants in pots, which are generally not limited by light, water, or nutrients. My results showed that root proliferation under field conditions, when it exists, is often less pronounced than in pot studies. Precision of forest species under field conditions in my study (from Chapter 4), calculated as root length density in N-rich patches minus control patches divided by the total root length in both patches, ranged from 0.18 to 0.41. However, precision of the three bottomland hardwood species (from Chapter 5) under aerobic conditions ranged from 0.37 to 0.62.

My data suggest that biomass growth of certain species (e.g. *Fraxinus pennsylvanica*, *Nyssa aquatica*) was greater when nutrients were distributed heterogeneously compared to homogeneously. These results suggest that the spatial distribution of nutrients can affect the growth of individual plants and may affect net primary production (NPP) when scaled to ecosystems. Future studies could examine the relative importance of the distribution versus the total amount of nutrients to ecosystem NPP.

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PUBLICATIONS:

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PROFESSIONAL PRESENTATIONS:

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Neatrour, M. A., R. H. Jones, and S. W. Golladay. 2004. Is there a tradeoff between root foraging for nutrients and soil anoxia in bottomland hardwood species? 2004 Ecological Society of America meeting. Portland, OR.

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