AN ANALYSIS OF THE ENERGETIC BUDGETS OF GRASSES TO ASSESS THE EFFECTIVENESS OF DIFFERENT COMPETITIVE STRATEGIES

by

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ABSTRACT

AN ANALYSIS OF THE ENERGETIC BUDGETS OF GRASSES TO ASSESS THE EFFECTIVENESS OF DIFFERENT COMPETITIVE STRATEGIES

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This study investigates how adaptation to a nutrient poor or nutrient rich environment affects efficient use of carbon belowground. Four methods compared nitrogen and carbon use in plants from a nutrient poor site (Hog Island, a Virginia barrier island) and a nutrient rich site (Konza Prairie, a Kansas tallgrass prairie): 1) a greenhouse study measured carbon and nitrogen allocation; 2) field observation of above and belowground costs; 3) root observation chambers assessed cost of individual roots and 4) turnover and response of microsite enrichment determined with minirhizotrons.

Andropogon gerardii (the dominant grass from Konza Prairie) and *Schizachyrium scoparium* (an important grass from Hog Island) were grown in Konza prairie soil (nitrogen rich) and dune sand (nitrogen poor) in a greenhouse. Watering system failure resulted in loss of all *A. gerardii* in sand. ^{14}C shifted belowground in *S. scoparium* grown in sand. ^{14}C:N exchange ratio was highest in *S. scoparium* in sand.

Aboveground biomass, C and N standing crops were higher than the belowground values. N concentration was highest in the 0-10 cm depth class. There were no significant differences by depth with C concentration. Root observation chamber results showed C:N ratio, C mm^{-1} and N mm^{-1} increased with width and age of individual roots. Individual N and C concentration differences were not significant. Neither site responded

significantly to microsite enrichment. Root length density (RLD), mortality and growth were lower for Hog Island (RLD 7.18±5.00, mortality 5.33±5.00, growth 3.87±3.71) than Konza Prairie (RLD 14.15±7.91, mortality 9.71±5.98, growth 10.82±5.52).

N and C concentration of individual roots exhibited no relationship with age or width, indicating no more storage of C or N in larger older roots than younger smaller roots. Individual root measurements should be used to calibrate N and C turnover measured with minirhizotrons. Roots need priming to elicit microsite enrichment response. *S. scoparium* dramatically shifted carbon allocation in nitrogen poor soil indicating plastic response to changing nutrient availability. *S. scoparium*'s plastic response to low nutrient environment suggests an active response necessary to survive transient catastrophic loss of a vital resource, water.

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INTRODUCTION

Traditionally, competitive influences have been researched from an aboveground perspective, largely ignoring the belowground portion of plants. In addition, previous research on roots has focused mainly on root/shoot biomass ratios (Bloom et al. 1985, Mooney and Winner 1991, Orians and Solbrig 1977, Thornley 1969) while little has been done on the physiological demand of roots. Current models rely upon root length rather than biomass to determine nutrient and water uptake rates (Baldwin et al. 1972, Cowan 1965, Cushman 1979, Gardner 1960) and at least one study found a grass species to have twice the mass per unit length of root of another closely related grass (Caldwell and Richards 1986).

Research which has observed root length density in natural systems has included observation of differences between competing species (Caldwell et al. 1981, Richards 1984), differences between two similar hardwood forests (Hendrick and Pregitzer 1993*a,b*), and the response to mass fertilization (Weber and Day 1996). Nutrient uptake rates of roots have been measured (Epstein 1976, Rastetter and Shaver 1992, 1995, Veen 1980).

Plants need and use both carbon and nitrogen. Energy is stored in the plant as carbon compounds. Plants acquire their carbon through photosynthesis in the aboveground portion of the plant. Nitrogen is a necessary building block of proteins and is acquired through the roots. Plants require carbon to build new roots, maintain existing roots and to actively transport nitrate into the root (Veen 1980, Lambers 1985, de Visser 1985, Van der Werf et al. 1988**,** Thornton et al. 1993, Bringham and Stevenson 1993,

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The journal model used for this dissertation is *Ecology*.

Penning de Vries et al. 1979, Poorter et al. 1991). The aboveground portion of the plant is, therefore, dependent upon the belowground portion for nitrogen and the belowground portion is dependent upon the aboveground portion for carbon (Bloom et al. 1985, Caldwell 1979, Orians and Solbrig 1977, Mooney and Winner 1991).

Competition and natural selection have resulted in the development of optimal strategies for carbon use. Endemic and dominant organisms of an ecosystem exhibit optimal strategies for carbon utilization within their environment. Light is the primary resource necessary for carbon acquisition aboveground. Nutrients and water are the primary resources acquired belowground. Nitrogen is the most common limiting nutrient in natural environments (Vitousek and Howarth 1991). The carbon cost for belowground production and maintenance must therefore be balanced by the carbon production benefit resulting from belowground resource acquisition.

The cost/benefit ratio of belowground carbon investment is dependent upon several factors. The cost for belowground investment consists of both a reduction in aboveground growth potential resulting in lower competitive effectiveness aboveground as well as the direct cost of carbon investment belowground. The strength of light competition therefore is an important factor in determining costs. The benefit of nitrogen depends upon carbon produced per unit nitrogen and per unit cost of nitrogen acquisition (Bloom et al. 1985).

Belowground Costs

A significant percentage of carbon fixed aboveground is allocated to belowground production. This carbon is used for maintenance of existing roots, new growth, and

uptake of some nutrients through active transport, particularly nitrate (Veen 1980, Van der Werf et al. 1988). In return for this investment, the aboveground portion of the plant receives nutrients and water. The belowground portion also stores nutrients and water and physically anchors the plant.

The belowground costs for a plant can be considerable (Caldwell 1979, Orians and Solbrig 1977, Van der Werf et al. 1988). The investment belowground can be observed from several perspectives. First, what is the ratio of carbon allocated belowground to that applied aboveground (root/shoot ratios)? Second, what is the cost per root based on measurements of carbon use by individual roots? Finally, models have been used to determine carbon flow to and carbon use by roots.

Root cost can be divided into a number of flows. These flows have been described in conceptual models, quantitative models and derived through empirical methods. A review of root costs by Caldwell (1979) divided belowground energetic demands into maintenance respiration, exudate production (including mycorrhizal costs), and root production. The maintenance of ionic gradients was placed under the category of maintenance respiration. Biosynthetic respiration should be proportional to biomass produced at about 0.24 g CO_2 g^{-1} biomass produced (Caldwell 1979). Bloom et al. (1985) also believed in a fairly constant cost per unit produced with a 20-30 percent range in carbon cost. Bloom et al.'s (1985) biosynthesis costs ranged from 520 to 712 mg C g-^{1} leaf. Bloom et al. added that constant biosynthetic cost was related to the inverse relationship between protein content and other energetically expensive constituents such as lignin. In contrast, maintenance costs can vary considerably between species. Although Caldwell (1979) cited a fairly constant rate of 0.4 mg of CO_2 g^{-1} dry weight hr^{-1}

for woody plants, he also cited rates for *Atriplex confertifolia* (Torr. & Frem.) S. Wats. (spiny saltbush or shadscale) from 0.01 to 0.1 mg CO_2 g^{-1} dry weight hr^{-1}. The costs of exudate production and mycorrhizae can be considerable. Caldwell (1979) cited respiration rates in pine trees twice that with symbionts than without. Roots therefore, place a significant, although necessary, demand upon the plant.

Competitive Plant Allocation Strategies

Plant ecologists have observed the relationship between plants in different environments and have proposed strategies to explain plant species dominance in different environments. These studies have resulted in plant strategy theories, primarily by Grime (Campbell and Grime 1992, Grime 1965, 1973*a*, *b*, 1974, 1977, Grime and Campbell 1991) and by Tilman (Gleeson and Tilman 1990, Tilman 1993, Tilman and Wedin 1991*a*, *b*, Wilson and Tilman 1991). These theories are based on a combination of observation of plant growth and of principles of population ecology and community ecology. Both Grime and Tilman drew heavily upon the r and K competitive strategies described by Pianka (1970). Meanwhile, plant physiologists have developed models for individual plants reliant upon economic analogies to explain carbon allocation patterns with the idea that plants maximizing their carbon returns is similar to the monetary returns used in economic models. Both methods have described development of similar morphologies and responses, although coming to the same conclusion from opposite directions. The community and population ecologists looked from the large scale to the individual; the plant physiologists looked from the molecular to the individual scale.

Grime (Campbell and Grime 1992, Grime 1965, 1973*a*, *b*, 1974, 1977, Grime and

Campbell 1991) divided plants into three different groups; ruderal (r-strategist), competitive (rapid and flexible allocation patterns) and stress tolerant (K-strategist). This theory hypothesizes that the best predictor for the dominant plant strategy is a combination of disturbance, resource availability, and competition. A complementary theory is the R* hypothesis proposed by Tilman (Gleeson and Tilman 1990, Tilman and Wedin 1991*a*, *b*, Wilson and Tilman 1991). This theory postulates the plant that is able to ultimately reduce limiting nutrients to the lowest concentration in monoculture will be the most successful.

Grime refined the r and K strategies by adding the competitive strategy for organisms and extending the description of the environment to predict the dominant species of an environment. r-strategists attempt to produce offspring in rapid bursts and, therefore, expect an immediate return on their investment. In contrast, K-strategists need to be nourishing parents while actively beating down their competitors. Survival for the K-strategist, therefore, means killing off competition rather than overwhelming the competition with sheer numbers of offspring (Pianka 1970).

Grime (1977) and Bloom et al. (1985) suggested that plants adapted to low nutrient conditions (Resource Poor or RP plants) are no more efficient at acquiring nutrients at low concentrations and that the importance of competition belowground declines in low nutrient environments. Successful plants in low resource environments are, therefore, stress tolerant rather than specially competitive in a stressed environment. In addition, Grime (1977) stated that high phenotypic plasticity is disadvantageous in environments with extreme environmental stress. Grime (1977) further described traits of stress tolerant plants. The relevant strategies of plants in this study included small or

leathery leaves, long lived recalcitrant leaves and roots, perennial life history, and a small proportion of annual production devoted to seed production (Table 1). In essence, the stress tolerant plant is a K-selected species (Pianka 1970).

TABLE 1. Characters of plants adapted to resource rich and resource poor environments. Described by both Tilman and Wedin (1991 a,b) and Grime (1977).

Character	Resource Rich (RR) plant	Resource Poor (RP) Plant
Litter	Copious, often persistent	Sparse, sometimes persistent
Maximum growth potential	Rapid	Slow
Life form	Perennial	Perennial
Turnover (above and belowground)	Short	Long
Shoot growth	Peak production period	Evergreen
Plasticity of response	High	Low
Root/shoot ratio	Low	High
Palatability	High	Low

Tilman's R* hypothesis (Tilman and Wedin 1991b) is based upon field experiments; it provides a tight mesh with Bloom and Grime's theories on optimal plant strategies. The essence of his theory is "that the most important determinant of nutrient competitive ability of a species is the concentration (called R*) to which the limiting nutrient is reduced in a steady-state, nutrient limited monoculture". The observable plant traits are the same as those described by Bloom et al. (1985) and Grime (1977) (Table 1). R* may be an integration of the successful traits for a resource limited site. In essence, if an organism optimally forages for a resource it will reduce the resource down to its minimum. This theory was based primarily upon the idea that plants able to outstrip the environment of resources will prevent the success of other plants. This contrasts with

Grimes's idea that stress tolerant species survive not through competitive interaction but through survival and implies that belowground competition may actually increase with decreasing resource availability.

Individual Plant Models of Allocation

While Grime, Tilman and others worked on competitive strategies from the perspective of community and population ecology, plant physiologists worked to develop models of individual plants based upon economic analogies. These included the more mechanistic approaches by Orians and Solbrig (1977), Caldwell (1979) and Mooney and Winner (1991). Their attempts originated not from field observations, but from theoretical optima derived from economic models of cost/benefit ratios.

Bloom et al.'s (1985) and Caldwell's (1979) economic analogies allowed the authors to establish relationships between carbon and resources important to the plant and to more carefully consider turnover and growth rates. Table 2 lists the economic terms and the ecological correlates used by Bloom et al. (1985). Most models of concern focus upon carbon as the primary resource of interest. Nitrogen or water then become secondary resources required for carbon increases. Profit is therefore net carbon gain. Nitrogen or water, because they are the most likely to be limiting, are considered resources which limit profit. Profit, net carbon gain per unit time, is the goal for which most of these models are optimized.

TABLE 2. Economic and ecological definitions of terms (from Bloom et al. 1985).

Term	Economic Definition	Ecological Definition
Process	Necessary business function, e.g. manufacturing or marketing	Necessary biological function, e.g. growth, maintenance, or defense
Resource	Raw materials required for a process, e.g. steel or labor(g or h)	Materials in the environment or within the plant required for a process (g of C,N or H_2O)
Reserve	Internally stored resource (g)	Internally stored resource (g of C,N or H_2O)
Product	Goods or service (g or h)	Biomass (g of C,N or H_2O)
Supply	Availability of a resource at the site of a process (g/hr or hr/hr)	Availability of a resource at the site of a process (g/hr of C,N or H_2O)
Demand	Requirement for a resource at the site of a process (g/hr or hr/hr)	Requirement for a resource at the site of a process (g/hr of C,N or H_2O)
Cost	Money spent per unit resource ($/g or $/hr)	Reserves expended to increase the supply of a resource (e.g. g C expended / g N acquired)
Revenue	gross income ($)	Gross resource gain (g of C,N or H_2O)
Profit	revenue minus cost ($)	Net resource gain (g of C,N or H_2O)
Marginal Product	Change in production per change in resource supply (δg product/δg resource)	Growth response to change in resource supply (δ g biomass/δ g of C,N or H_2O)
Exchange Ratio	relative cost of two resources ($/$)	Relative quantities of two resources acquired per expenditure of resource (e.g. g C /g N per expenditure of g H_2O)
Marginal Rate of Technical Substitution	Increase in supply of one resource necessary to compensate for loss of another and still maintain the same production level (δg/δg,δhr/δg, or δhr/δhr)	Increase in supply of one resource necessary to compensate for loss of another and still maintain the same growth rate (e.g. δ g C/δ g N)

Bloom et al. (1985) and Caldwell (1979) both stated that nutrient and water cost of growth is optimally balanced when their benefit is balanced. Plants should adjust allocation so that all resources equally limit growth until the ratio of the marginal product to cost is equal for all resources. This analysis led to some interesting ideas. Bloom et al. (1985) believed that total maintenance respiration integrated over the tissue lifetime may not vary greatly among tissues and species. Nutrient cost of growth increases at high nutrient availability. Plants therefore allocate more resources belowground in nutrient stressed environments to provide a carbon/nutrient balance. Photosynthesis and nutrient uptake are adjusted to maximize rate of acquisition of specific limiting resources. Once again, plants adapted to poor resources (RP plants) are less plastic in allocation patterns than plants adapted to rich resources (RR plants). Prolonged tissue life maximizes resource use in resource poor environments. This results in a relatively high carbon gain per unit nitrogen similar to those found in nutrient rich sites.

Orians and Solbrig (1977) developed a cost income model to explain partitioning patterns of plants with an emphasis on plants adapted to water deficit conditions. Their studies found that water loss was correlated with photosynthetic rate. Xerophytic leaves produce reduced returns per unit compared to mesophytic leaves and may also be more costly to construct. There is a higher root/shoot ratio with xerophytic plants. Plants with xerophytic leaves also have longer lived roots and hence lower turnover. Mesophytic leaves reach the break even point (cost to benefit ratio) much sooner than xerophytic leaves. There is an inverse relationship between ability to photosynthesize and ability to extract water. There is a point where the water potential drops below the threshold of the ability of the leaves to photosynthesize. This occurs sooner in the mesophytic plant than

in the xerophytic plant. The mesophytic plants are therefore unable to photosynthesize under low water potential while the xerophytic plant is still able to photosynthesize. This results in higher returns even though under optimal conditions the mesophytic leaf has a higher photosynthetic potential. Orians and Solbrig (1977) believed that the net energy gain per unit time is a key component of fitness in plants.

Mooney and Winner (1991) argued for a set point of carbon to nutrients to determine growth rates and partitioning. That is, there is an ideal carbon to nitrogen ratio (set point) controlling allocation. This hypothesis has three parts. 1) Plants adjust their root/shoot ratio under changing resource availability. 2) In doing so, they maximize their growth rate. 3) The mechanism for controlling root/shoot partitioning is related to the internal ratio of carbon to nutrients of specific plant organs. According to this theory, carbon partitioning may be controlled through either or both carbon or nitrogen control. In carbon control, source loading by converting excess carbon to starch in the leaves reduces carbon allocation belowground. Mooney and Winner (1991) hypothesized that under carbon limitation (shading) starch synthesis has priority over sucrose synthesis, thus leading to decreased flow of carbon to the roots. Alternatively, nitrogen levels can be controlled in a similar manner from the roots. The balance of carbon to nitrogen would preferentially allow carbon to accumulate in the roots while maintaining the same photosynthetic rate. Mooney and Winner (1991) stated that the nitrogen content of leaves is positively correlated with nitrogen content of the soil. There is also a similar relationship between nitrogen and leaf tissue dry matter and growth rate. They stated that "it follows then that a given species, which has evolved in a given resource matrix, will have a genetically controlled partitioning schedule that ensures maximal growth for the

prevailing environmental conditions". The nitrogen control theory seems to mesh with several physiological models of nitrogen and carbon allocation. In support of Mooney and Winner's (1991) theory, nitrogen may exist in a dynamic pool within a plant. The roots may control the aboveground portions through a combination of source sink relations and hormonal control. Cooper and Clarkson (1989) proposed a method of nitrogen transport which places most of the nitrogen within a cycling of amino-N pool in the xylem and phloem. The difference between the need of an organ or the availability of nitrogen to a root will therefore influence uptake rate.

Direct control of the aboveground portion of the plant can be accomplished through a combination of hormonal actions and source/sink interactions. Davies and Zhang (1991) proposed that control of photosynthetic rate, at least in drought conditions, is controlled by the roots. Several studies suggest that ABA acts as a possible control mechanism at least in some species. The primary lines of evidence were obtained from split pot studies in which half the roots of a plant are water stressed. Water potential of these plants was maintained but half of the leaves senesced even though they received adequate water. Separately, control of stomata was accomplished through transport of ABA from the roots. Wyse (1986) discussed the physiology of carbon transport with an emphasis on the source-sink relationship as a means of control. In short, apoplastic sucrose levels are maintained at the sink to increase or maintain carbon flow through the phloem. This suggests that roots may actively control flow of carbon in the plant.

The work of Orians and Solbrig (1977), Mooney and Winner (1991) and others resulted in the development of carbon flow models of individual plants in an attempt to better understand the importance of nutrients and carbon in the success of individual

plants. In order to build these models, the value of carbon and nutrients had to be addressed as did empirically determining the partitioning of carbon, not only within the whole plant, but within individual organs.

Two main strategies for mathematical models of nutrient allocation within plants have been used. The first is a strictly derivative method akin to the approach used in developing the Lotka-Volterra equations. This method was used by Thornley (1969, 1972), Caldwell (1979), and Sharpe and Rykiel (1991). In this case, the assumption was that the plants maximized growth while maintaining a uniform C to N ratio as suggested by Mooney and Winner (1991). The alternative to these models is to develop empirical models of root and shoot partitioning. In this technique, compartmental models are developed in which flows are dependent upon compartment size. This system is easier to develop but does not allow for a derivation to determine optimal conditions.

Sharpe and Rykiel (1991) attempted to develop a mechanistic model of carbon partitioning in plants using the general theories of allocation found in Bloom et al. (1985). Carbon is allocated to organs that enhance the acquisition of resources most severely limiting growth. They postulated that RR plants would be more flexible in resource allocation patterns than RP plants and that when presented with sudden increases in resources the RP plants would respond by increasing storage. This would result in increased root/shoot ratios for RP plants.

Thornley (Brugge and Thornley 1985, Johnson and Thornley 1987, Thornley 1969, 1972) developed several mechanistic models dependent upon the Michaelis-Menton kinetic with carbon and nitrogen as the substrate and uptake or photosynthesis as the enzymatic reaction. The models were relatively simple and employed two or three

compartments, two of which were roots and shoots. Flows in the final model were interdependent and maximized carbon acquisition. The results reflected those findings from others that root/shoot allocation declined with increased nitrogen uptake and increased with increased carbon acquisition. In this model, both roots and shoots controlled allocation. This is similar to the description of control by Orians and Solbrig (1977).

Spek and Oijen (1988) developed a model of root and shoot growth that divided root and shoot activities into growing shoot parts, mature shoot parts and roots. There were sub-compartments based upon the form of carbon and nitrogen (nitrate, amino acids, structural nitrogen, structural carbon, and soluble carbohydrates) with appropriate flow both between organs and forms. The model used Michaelis-Menton equations to describe most flows. The model was tested against corn grown in containers. The result of the experiment was that NO_3 uptake rate was dependent upon C concentration. Low nitrate resulted in higher root/shoot ratios; split pots resulted in moderate root/shoot ratios. The split root system also had the highest root biomass. Structural carbon is preferentially provided to the side of the system which has low nitrate concentration in the split pot system. Nitrate uptake per gram was highest in the higher nitrate system and in the higher nitrate side of the split pot system. These results in the split pot system were due to differences in C concentration in the sink/source.

The individual root models were effective in determining steady state C allocation patterns, however, they ignored spatial and temporal heterogeneity. These can be vital in determining costs for nitrogen acquisition. If a resource is ephemeral, attempts to retrieve it through root growth will result in a higher cost per unit or resource. Maximizing

profits therefore requires optimization to the heterogeneity of resource availability.

Objective and Hypotheses

As has been discussed, most conceptual models of plant strategies agree on the resulting strategies for low nutrient conditions. However, the different models do have some disagreement over the actual forces involved between species that make a species more fit for a particular environment. While Grime (1977) believes that belowground competition declines in low nutrient environments, Tilman and Wedin (1991b) believe that belowground competition drives the systems selective pressures. Most mechanistic models optimize growth per unit time; however, some have optimized other factors. The objective of the current study was to determine whether plants adapted to sustained life in nutrient depauperate environments (RP plants) use carbon more conservatively than plants adapted to nutrient rich environments (RR plants) by observing the mechanistic processes of the plants. Specifically, it should be more energetically efficient to use carbon conservatively in a nutrient depauperate environment. Conversely, plants adapted to nutrient rich environments should rapidly remove nutrients from the surrounding environment. The conservative or aggressive use of carbon resources can be deduced from; 1) carbon and nitrogen costs, 2) exchange ratios, and 3) carbon allocation patterns (Table 3).

If competition drives plant response to the environment one might expect that carbon would be allocated to the portion of the plant which was being most severely

TABLE 3. List of hypotheses.

	Hypothesis	Measure	Experiment
1) Costs (C and N)	a) N cost (g N/g C) is higher for RR plants	C/N ratio	Field Greenhouse
	b) % N (dry weight) is higher for both aboveground and belowground biomass in nutrient rich environments	% N	Field Greenhouse
	c) C and N cost per unit root length is higher in RR plants	C mm^{-1}, N mm^{-1}	ROC†
	d) More storage of nutrients and carbon resulting in larger difference in % N and % C with root age for nutrient poor site	% N by width % N by age % C by width % C by age	ROC Greenhouse
2) Exchange ratios (units of resource required to acquire unit of another resource)	There is a lower carbon expenditure per unit N recovered aboveground in native environment.	% ^{14}C roots N^{-1} aboveground Δ C roots N^{-1} aboveground	Greenhouse
3) Carbon Allocation	a) Relative C allocation belowground is higher for RP plants	^{14}C to roots Δ C below / Δ C total	Greenhouse
	b) belowground biomass is higher in resource rich environments	biomass (g $m^{-2)}$	Field Greenhouse
	c) response to microsite high resource additions is greater in resource rich environments	mortality growth	Minirhizotrons (nutrient addition)

†Root Observation Chamber

impacted. Table 4 shows all possible interactions between aboveground and belowground competition. Because resource-rich environments typically have high stem densities it is generally accepted that aboveground (light) competition is high. Low aboveground competition is therefore unlikely. Likewise the high light penetration due to low stem density found in resource poor environments implies low aboveground competition. Unfortunately, the evidence for belowground competition is not as clear.

TABLE 4. Potential effect of competition on allocation of carbon.

	Competition		Investment	
	Aboveground	Belowground	Aboveground	Belowground
Nutrient Rich	High	High	⇑	⇑
	High	Low	⇑	⇓
	Low‡	High	⇓	⇑
	Low‡	Low	⇓	⇓
Nutrient Poor	High§	High	⇑	⇑
	High§	Low	⇑	⇓
	Low	High	⇓	⇑
	Low	Low	⇓	⇓

‡ Condition unlikely due to high stem density, § Condition unlikely due to low stem density

As has been shown, proportionally more carbon is allocated belowground by RP plants. This does not, however, address the question of whether competition occurs to a significant degree belowground. Andrewartha and Birch (1953) stated that

> "Competition occurs whenever a valuable or necessary resource is sought together by a number of animals or plants (of the same kind or of different kinds) when that resource is in short supply; or if the resource is not in short supply, competition occurs when the animals or plants seeking that

resource nevertheless harm one another in the process."

Competition, therefore, involves the removal of a limiting resource required by competing individuals or by direct harm caused to another. Because the current study was not looking at allelopathy, I focused upon the former rather than the latter form of competition. Because both individuals are competing for the same resource and they are likely to be unequally adept at gathering the limiting resource, one individual will successfully garner more resource than its competitor. If belowground competition is occurring, resources must be acquired either so quickly that another individual must work harder and therefore expend more carbon per unit resource or it must reduce the resources to the point where no others can survive. In actuality, a little of both of these actions is likely to occur. These processes can be seen in the exchange ratios (Table 3). As stated by Bloom et al. (1985), nutrient exchange ratios should be at their minimum when an organism is within its native environment (hypothesis 2). Nutrient exchange ratios were determined in a greenhouse study in which both nitrogen and carbon acquisition were quantified for both a grass adapted to high resource availability (RR plant) and a grass adapted to low resource availability (RP plant).

Grime's plant strategy theory perceives the RP plants as passive survivors. Stress tolerant individuals survive in an environment so depauperate that no others can survive. In this view, competition does not occur between stress tolerant individuals. In contrast, RR plants are fiercely competitive. If this is so, RR plants should not be able to survive in a resource poor environment even if they are grown without competitors. In contrast, Tilman's R* hypothesis views RP plants as actively depriving others of soil resources by

depleting the most limiting resource below the threshold of potential competitors. The R* hypothesis, therefore, proposes that competition may actually increase belowground in resource poor environments. Additional properties described by both Tilman and Grime include information on the costs of the above and belowground components. The costs include the (hypothesis 1a) N cost per unit carbon, (hypothesis 1b) percent nitrogen and (hypothesis 1c) carbon and nitrogen per unit length. Among the traits that Grime described for survival in nutrient poor environments was the increased storage of both nutrients and carbon (1d); this was also discussed by several individual plant model studies (Orians and Solbrig 1977, Spek and Oijen 1988, Sharpe and Rykiel 1991, Brugge and Thornley 1985, Johnson and Thornley 1987, Thornley 1969, 1972 **)**.

In resource rich environments, competition belowground is more difficult to test because light may be the most limiting resource. However, limiting nutrient residence times may be shorter and more heterogenous due to the nature of the soil matrix. Because sand holds resources so poorly, the resources are likely to be either concentrated but extremely ephemeral or diffuse but evenly distributed. In contrast, silts and particularly silicate clays are more likely to retain their resources. In addition, the resources are likely to be attached to organic matter within the soil and to be relatively concentrated in pockets (Jackson and Caldwell 1993, Gupta and Robinson 1975).

Because the nutrients in these pockets are so rich, and because aboveground production is linked to belowground resource allocation, it may be more energetically productive to use carbon to quickly acquire nutrients before a competitor can acquire them. Exploration and exploitation are more important in this environment. Belowground competition therefore takes the form of effective exploration and

exploitation of nutrient pockets. This can be observed as increased C allocation and higher turnover. This may not lead to higher root/shoot ratios because a diffused presence in the soil matrix is less important than the ability to use resources to quickly deploy into nutrient pockets. The diffusive strategy of plants adapted to low resource availability results in low turnover but high presence. In effect, both systems may be allocating a large proportion of their carbon resources belowground. However, the carbon is used in bursts for the RR plants while it is a constant draw in the RP plants.

Although the current study did not directly address the competitive interaction of plants, it used competitive interactions to predict carbon flow and nitrogen acquisition. The balance of these flows determines the competitive ability of the individual plants in a given environment. There is likely to be high competition in all environments belowground because there is almost always a soil resource limiting production. In addition, there is a need to hinder competitors through either exhaustive depletion or removal of the quick and easy resources leaving only the more costly resources. RR plants should therefore have higher mortality rates (hypothesis 3c), as has already been described by several other researchers (Grime 1977, Caldwell 1979, Tilman and Wedin 1991*b*) and a more rapid response to microsite nutrient additions (hypothesis 3c). In addition, although absolute biomass belowground in the nutrient rich site should be higher than in the nutrient poor site (hypothesis 3b), the relative allocation belowground should be higher in the nutrient poor site (3a) (Grime 1977,Tilman and Wedin 1991*b*).

To summarize, it is proposed that plants adapt to their environments by maximizing limiting resource acquisition efficiencies. The efficiency with which a resource is acquired relates to both the quantity and ephemerality of the resource. Light

may be limiting in a terrestrial system, and therefore may limit carbon acquisition. The light limitation, however, is due to light competition from others and requires the acquisition of belowground nutrients to allow growth to overgrow competitors. Terrestrial plants, therefore, require an acquisition strategy that balances allocation belowground to acquire nutrients and allocation aboveground to acquire carbon. These hypotheses assume that the resource poor environments have steady, low resource availability and the resource rich environments have ephemeral high resource availability. It is also assumed that the resource availability is more heterogenous in the resource rich environments. RR plants should maximize nitrogen acquisition through rapid ephemeral expansion of the root system because there will be a significantly higher return of nitrogen per unit carbon. Because of the low return of N in carbon investment, RP plants will also maximize nitrogen acquisition by allowing a greater proportion of the carbon to be placed belowground and increasing their nitrogen use efficiency at the cost of productivity.

The purpose of this study was to determine how adaptation to a nutrient poor or a nutrient rich environment affects efficient use of carbon belowground. The strategies of the plants used in this study were measured by estimating the efficiencies, costs, and exchange ratios through four methods. As discussed earlier, costs of an asset affect the rate at which its value can be recouped through its use. The cost in terms of C and N of above and belowground tissue was measured both in the field and in the greenhouse. It is expected that plant tissue costs for the nutrient rich environment will be higher than those for the nutrient poor environment. To determine if the cost of roots changed with age, individual roots of known ages were sampled and analyzed in the field. Because wider,

older roots are expected to be the site for nitrogen and carbon storage, a higher concentration of carbon and nitrogen would indicate storage was occurring in the individual roots. It is expected that more storage should occur at the nutrient poor site. To determine the response of the system to dynamic nutrient addition, nitrate and ammonium amended solution was added to the side of minirhizotron tubes and the response in absolute root lengths as well as root dynamics (growth, mortality, longevity) was measured with minirhizotron tubes. An expected response to nitrogen enrichment is increased growth and mortality for the nutrient rich site and increased growth and longevity for the nutrient poor site. A greenhouse study was performed to directly measure carbon transport to roots in order to determine the exchange ratio of C and N and therefore quantify the cost and benefit of roots. Plasticity of response was also measured by growing the plants in both native soil and either a nutrient rich or nutrient poor soil. It is expected that plants adapted to an environment are more efficient at acquiring nitrogen in their environment than plants adapted to a different nutrient availability regime. By measuring the cost, benefits and longevity of resources as well as their response to a small scale stimulus (microsite fertilization) and a change in their native nutrient regime, it was hoped that the native efficiencies of the plants could be assessed.

METHODS

I used microeconomic concepts of cost/benefit analysis to determine effective strategies within high and low nutrient conditions. By building roots, a plant is taking carbon away from the production of aboveground biomass which generates carbon through photosynthesis. Cost was equated to carbon sent belowground as roots and aboveground as leaves. Aboveground tissues provide a direct return in investment through photosynthesis. In contrast, roots are 'non-productive' but provide an indirect return through the provision of resources necessary for photosynthesis. This investment in a 'non-productive' asset must provide resources which increase the production aboveground beyond the cost of the belowground asset for it to be an effective strategy.

Cost must be integrated over the life of an asset. Cost includes, the cost of construction and the rate of construction of the asset. The construction cost is estimated by sampling biomass and determining the carbon and nitrogen content of the tissue. The rate of construction and durability was determined by minirhizotron observation as the growth rate and mortality of roots. Integrated cost is the cost per unit multiplied by the number of units produced over a given period of time (g C / month).

For a given asset there is a return from the investment (profit). The profit/loss is the change in carbon aboveground. The durability of an asset affects the integrated return expected from a given asset. If an asset has a high cost and a high return, the durability of the asset can be shorter and still maintain a profit. If the asset has a lower return, the durability must consequently be longer. The integrated return is the profit that results from the resources provided by a given asset for the lifetime of that asset.

The above measures assume that the systems are static. Experiments were also performed to determine whether the response of plants to resources were plastic. Two aspects were tested. The first was an experiment in which an ephemeral nutrient source was added to plants in their native environment. Possible responses included quick growth in regions to which the resource is added, and a lack of response. Although a quick response improves the likelihood that an individual will acquire the resource, if the resource provides an integrated return less than the cost of building the belowground asset the expenditure would have been a loss. The second experiment was on individual plants to determine whether there was a change in resource allocation when the plants were grown in a soil other than their native soil.

Study sites

Two sites were chosen for this study. The first site was a nitrogen poor, sand dune ecosystem located on Hog Island Virginia, part of the Virginia Coast Reserve LTER site. The second site was located in the nutrient rich, tall grass prairie of the Konza Prairie LTER site. Both nitrate and ammonium are much higher in the Konza Prairie site (180 mg NO_3/kg, 210 mg NH_4/kg) (Turner et al. 1997) than in the Hog Island site (0.28 mg NO_2-NO_3/kg, 2.54 mg NH_4/kg) (Conn and Day 1996). Both sites are at approximately the same latitude. Hog Island is located at 37.3 degrees N and Konza Prairie is located at 39.1 degrees N (Fig. 1).

Long term weather data were not available from the sites themselves. Nearby sites were therefore used to calculate long term means. For Hog Island, a station located immediately across the lagoon in Painter, Virginia was used as the surrogate station. The

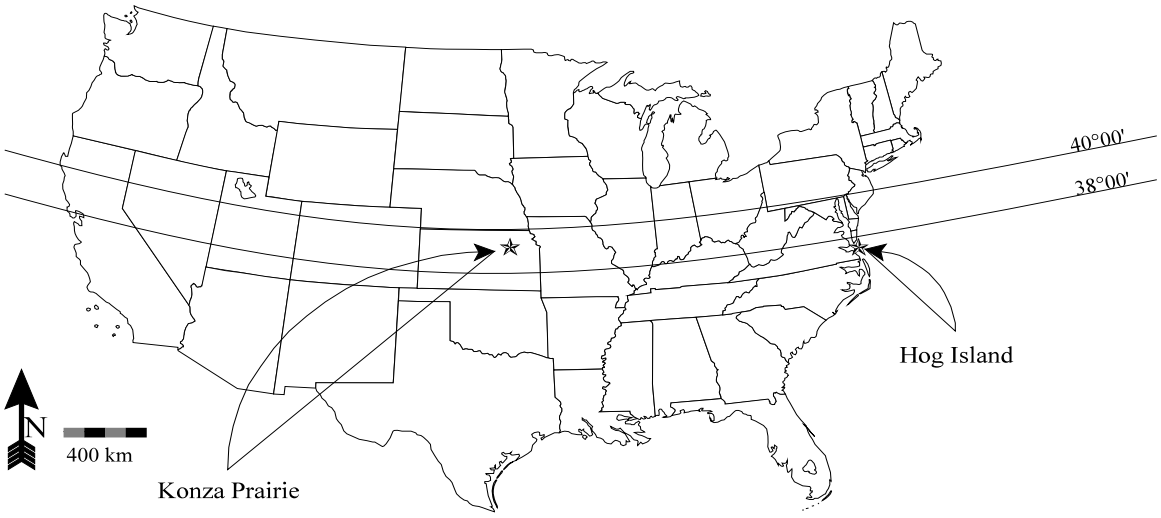

Figure 1. Map showing location of research areas.

surrogate station for Konza Prairie was in Manhattan, Kansas, 8 to 16 km from the research site. Regressions of monthly mean temperature and monthly total precipitation between Konza Prairie and Manhattan Kansas yielded R^2s of 0.99 for mean monthly temperature (May 1982 to March 1985) and an R^2 of 0.85 for monthly total precipitation (April 1982 to November 1985) (Bark 1997). The Virginia Coast Reserve LTER site, of which Hog Island is part, is younger and had considerably more problems with its weather station. The comparison data are therefore of shorter duration and missing months due to equipment failure. Temperature data are available from December of 1989 to December of 1994 with some small data gaps for both sites ($R^2 = 0.98$). Unfortunately, a faulty rain gauge resulted in only 8 months of data for comparison of precipitation from March of 1994 to December of that year ($R^2=0.72$). Data for the R^2 calculations of Hog Island and Painter were obtained from the Virginia Coast Reserve data web page.

The temperature profiles are similar although Konza is warmer in the summer and drops below freezing more frequently in the winter months (Fig. 2). 1998 was a fairly

typical year for Konza while Hog Island experienced a particularly warm summer. Thirty year mean total precipitation occurs highest in the summer months for Konza while the precipitation is more erratic for Hog Island (Fig. 3). Mean total precipitation is higher on Hog Island (1065 mm) than on Konza Prairie (859 mm). Adjusted potential evaporation increases with temperature and consequently is higher in the summer months. The resulting soil moisture deficit occurs in the summer (Greenland and Hayden 1997, Bark 1997). In 1998, Hog Island had a wet spring followed by a dry summer while Konza Prairie had a wet spring followed by a moderate to wet summer. July was particularly wet and August was particularly dry in Konza Prairie.

The soils at Konza are pachic argiustolls (Jantz et al. 1975) with a deep organic rich A horizon and a high clay content. In contrast, the Hog Island site has poorly developed deep mineral soils (udipsamment) with a high sand content (Newhan-corrolan complex) (Dueser et al. 1976). In addition, the Hog Island site has a high salinity input from the nearby ocean which was absent at the Konza Prairie site.

Both sites are dominated by perennial grasses although the prairie site has a much higher mix of herbs and forbs (Briggs and Knapp 1995). The Hog Island site is dominated by *Ammophila breviligulata* Fernald (American beach grass), *Spartina patens* (Aiton) Muhl. (salt hay grass), *Panicum amarum* Ell. (Atlantic coastal panic grass), and *Schizachyrium scoparium* (Nash) Gould (little bluestem) (Dilustro and Day 1997). Konza is dominated by *Andropogon gerardii* Vitman (big bluestem), *Sorgastrum nutans* (L.) Nash (indian grass), *Schizachyrium scoparium* (Nash) Gould (little bluestem), and *Panicum virgatum* L. (switch grass) (Briggs and Knapp 1995).

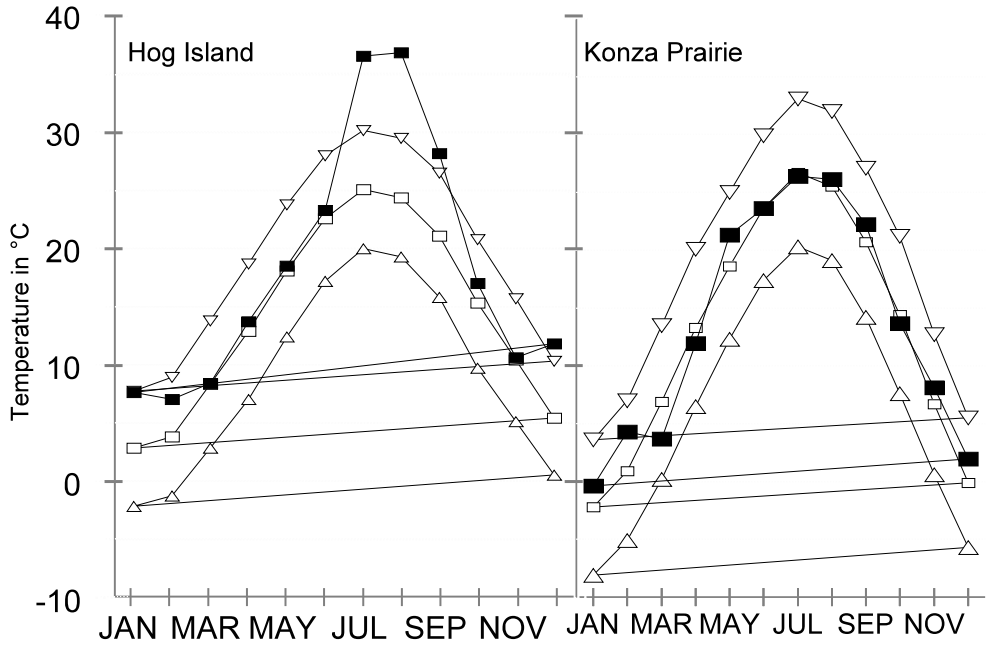

Figure 2. Temperature for Hog Island and Konza Prairie (1961-1990). □ = mean of mean temperature, ■ = 1998 mean temperature, ▿ = mean maximum temperature, △ = mean minimum temperature. Actual data are from Manhattan, Kansas and Painter, Virginia meteorologic stations (Long term data from Greenland and Hayden 1997, Bark 1997)(1998 data from LTER data servers).

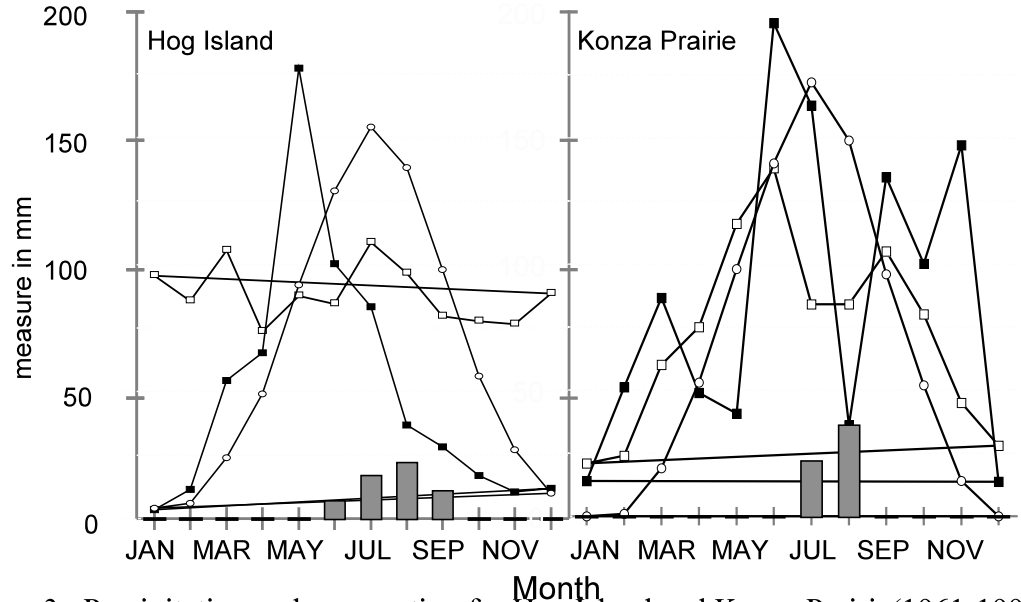

Figure 3. Precipitation and evaporation for Hog Island and Konza Prairie(1961-1990). □ = mean precipitation, ■ = 1998 precipitation, ○ = mean Adjusted potential evapotranspiration, Bar = mean Soil Moisture Deficit. Actual data are from Manhattan, Kansas and Painter, Virginia meteorologic stations (Long term data from Greenland and Hayden 1997, Bark 1997)(1998 data from LTER data servers).

Both sites have significant regular disturbance events. The Hog Island community was located on a 42 yr old sand dune at the time of the study. As are most barrier islands, the landscape is extremely dynamic with rapid island building and erosion (Shao et al. 1998, Greenland and Hayden 1997). Large scale accretion and erosion events of Hog Island occur frequently and are usually associated with storms. Storms also cause disturbances such as over-wash events. The prairie site is a fire dominated community. Without fire the species composition changes relatively rapidly (Abrams et al. 1986, Briggs and Knapp 1995). The site chosen for the study was normally burned at a 2 year interval and was burned both in the late winter/early spring of 1997 and 1998. The burn prior to 1997 was in 1995.

Barrier islands provide a particularly good environment to observe nutrient-limited roots. The sandy soil of the islands makes root observation relatively easy and the low nitrogen status of the soil makes nitrogen availability easy to manipulate. Although observation of roots is more difficult due to the heavy soil texture, tall grass prairie sites provide a good example of a naturally nutrient rich grassland site.

Experimental Design for Field Studies

The two sites had different restrictions for site selection, and as a result there were slightly different plot placement strategies. Both sites received three root observation chambers for individual root collection and 12 minirhizotrons. Hog Island had 9 clip plots and cores while Konza had 10 of each. The soil cores were located within the clip plots. The placement of all chambers, minirhizotrons, and sample plots was randomly determined. The minirhizotron and chamber orientation were also randomly determined.

The 35 year old sand dune of Hog Island is broken up and surrounded by *Myrica cerifera* L. in the swales. As a result, islands of grass arise surrounded by a tangle of wax myrtle. To ensure that the grass ecosystem was measured instead of the surrounding *Myrica cerifera* L., I used three separate grassland patches, each with a minimum 3 m buffer with the *Myrica cerifera* L., and distributed the treatments between them (Fig. 4). The Konza study site was restricted to a single area. All treatments, therefore, were located within this single area (Fig. 4).

Field measures of carbon, nitrogen and biomass

Static costs in terms of carbon, nitrogen and biomass both above and belowground were determined with clip plots and soil cores. The samples were taken on 9/17/98 for Hog Island and 7/29/98 for Konza Prairie. Although the Hog Island samples were taken relatively late in the season, Dilustro and Day (1997) found that there was no significant difference in aboveground biomass from August through October. Vegetation from quarter meter square clip plots, located as described above, was oven dried at 80°C for 24 hours and divided into live and dead. The samples were then weighed and ground in a Wiley mill. Subsamples of large samples were ground a second time in a wigglebug. Carbon and nitrogen levels were established with a Carlo Erba ea1108 Elemental Analyzer set up with a proportional volume of tungsten oxide to reduced copper wire in a single column. The regression method was used to determine C and N values with sulfanilimide analytical standard. The samples were divided between two runs.

Soil cores were taken from the center of each clip plot. Each soil core was 30 cm

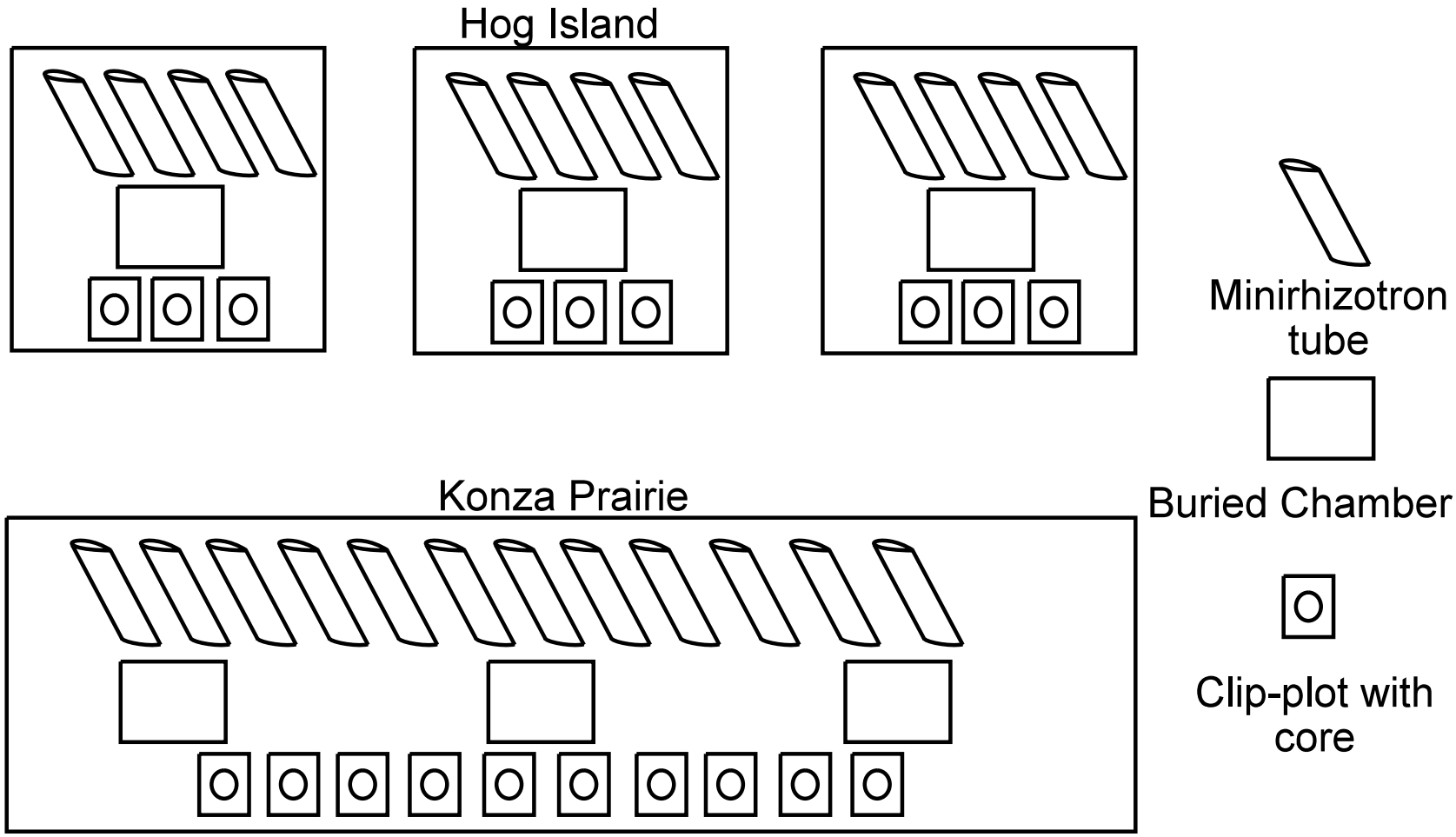

Figure 4. Design of field experiment.

deep and divided into three 10 cm depths. The cores were refrigerated until processing was complete. Processing for the Konza soils consisted of hand picking the cores, running the remaining soil as well as the picked roots through a hydropneumatic elutriator, and hand picking the roots from the organic matter. Processing the Hog Island cores involved running the cores through the hydropneumatic elutriator and hand picking roots from the resulting organic matter. The roots from both sites were washed from the soil in late October of 1998. Root lengths were obtained with a Decagon root analysis system. Because the roots were very fine, the highest level of magnification was used and many cores, especially from the Konza site, required processing root lengths in batches. Root lengths were obtained in May and June of 1999. After root lengths were obtained, the roots were dried, weighed, ground and analyzed as described above for the clip plots. Smaller samples were not ground or were exclusively ground in the wigglebug due to fear that too much sample would be lost.

In coordination with the soil cores, separate cores were taken to establish soil water content. The cores were divided into 3, 10 cm depth classes and the sampling followed the same design as the clip plots. The cores were placed in pre-weighed bags (weighed after oven drying). The wet cores were then weighed, dried at 100°C for 24 hours and weighed again. The difference in weight divided by the wet weight of the soil was the percent water content.

Root growth, mortality and microsite enrichment

Minirhizotrons were used to measure root growth and mortality as well as to measure the response of roots to microsite enrichment. Minirhizotrons are clear tubes

through which roots can be observed along their surface. The tubes used in this study were modified to allow for microsite enrichment along one side of the tube and observation along both sides. Frames were etched along both sides of the tubes and the bottom frame of each tube received a mark uniquely identifying each column of frames. Eight of the tubes were new and 16 were reconditioned tubes from a prior study. Reconditioning consisted of stripping the paint off the upper portion of the tube and polishing the tube to ensure a clear view. A channel was routed out of one side of the tube and a small clear acrylic tube was attached to the side (Fig. 5). The small tube was perforated prior to installation. The perforation allowed nutrient solution to be injected immediately adjacent to the observation frames. Each tube, therefore, had a treated and an untreated series of frames. The tops of the tubes used in Hog Island were painted black to exclude light. They were then capped with aluminum cans.

The Konza minirhizotron tubes were subjected to fire and special care therefore had to be taken in their preparation. A fire shield consisting of aluminum flashing wrapped with a fiberglass insulation liner was wrapped around a plywood disk. To prevent the fiberglass from entering the minirhizotron tube, a cap which fit inside the tube was manufactured out of PVC. The shield was placed over the unpainted minirhizotron tube so that it entered the soil around the top of the tube. No damage to the tube was observed upon sampling the tube after prescribed burns.

Tubes were installed at a 45° angle at both sites. On Hog Island, an auger guide was used with a soil auger to create a hole for the tubes. The tubes were installed in March 1998. A more intense effort was required at the Konza site. The installation occurred in December 1997 in several inches of snow and required the use of an

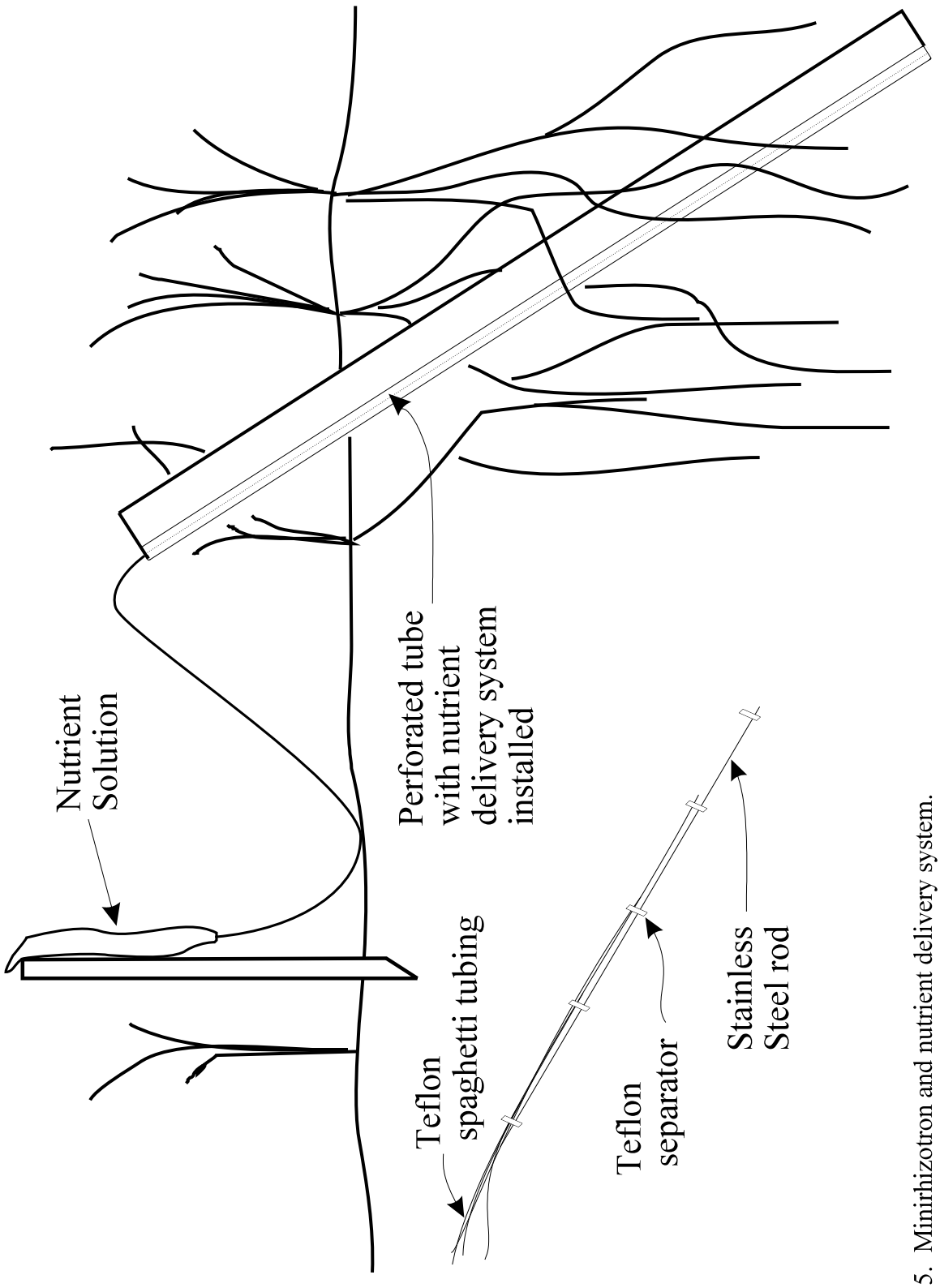

Figure 5. Minirhizotron and nutrient delivery system.

elaborate angle-iron jig. The holes were created by pounding in a sharpened automotive axle with a weight. The tubes were then pushed into the holes. A sledge hammer was usually required to pound the tubes into the holes to the desired depth. The excess tube length was then cut off with a hacksaw.

Two minirhizotron camera systems were used for this study. The minirhizotron camera has a cylindrical case which fits inside minirhizotron tubes. A hi-8 camcorder was plugged into the camera controller to record images along the surface of the tube. On Hog Island a battery pack was used with a Bartz minirhizotron camera. A small honda generator was used with the Konza prairie minirhizotron camera, also a Bartz camera. Although an indexing handle was available for the Konza system, it was not used.

A microsite enrichment system was used to slowly drip nutrients into the soil adjacent to one side of the tube. This system was constructed out of a stainless steal welding rod, Teflon spaghetti tubing, and a one liter sterile distilled water drip bag (Fig. 5). The sterile solution was enriched with one of three levels of nutrient: none, high and low. The no nutrient solution consisted of distilled water. The low nutrient level consisted of 10 times the ammonium and nitrate level found in the soil and the high consisted of 100 times the ammonium and nitrate levels found in the soil. The concentrations were determined assuming that the nutrient solution would infiltrate a region 2 cm in diameter along the length of the tube. This region would therefore have a volume of 314 cm^3. It was assumed the soil had a density of 1 g cm^{-3} and therefore weighed approximately one third of a kilogram. Field concentrations of nitrate and ammonium from Conn and Day (1996) and Turner et al. (1997) and the assumptions concerning the region affected by the nutrient solution were used to set the concentration

of nitrate and ammonium in the nutrient solution. The final concentrations (Table 5) were made from a mixture of ammonium nitrate, ammonium chloride and sodium nitrate. The 1 liter bags were then placed on stakes and the solution was gravity fed to the tubing in the microsite enrichment device. To ensure that the solution was evenly distributed along the tube, four shrink tube barriers covered in silicon were placed along the stainless steel rod. This system was tested with fluoresceine dye in a sandy loam using an ultraviolet light on the minirhizotron tube. There was a satisfactory distribution of the fluorescent solution along the treated length of the tube and no fluorescence on the untreated side of the tube. The solution took between 4 and 24 hours to be completely dispensed.

Table 5. Nutrient concentrations for microsite enrichment.

	Konza Prairie		Hog Island	
Nutrient	10x	100x	10x	100x
NO_3	600 mg/l	6 g/l	0.933 mg/l	9.33 mg/l
NH_4	700 mg/l	7 g/l	8.47 mg/l	84.7 mg/l

The minirhizotrons were observed at three dates (6/26/98, 9/11/98, 9/17/98 for Hog Island and 6/30/98, 7/23/98, 7/29/98 for Konza Prairie). Two weeks prior to the last minirhizotron measurement (Konza Prairie = 7/18/98, Hog Island = 9/3/98) the roots received a microsite enrichment treatment. Although there was a much longer initial period of observation for Hog Island, the period between the last two observations was similar. In addition, the purpose of setting the longer period was to divide the roots into long-lived and short-lived root classes. The older roots would be more likely to be used

for nutrient and carbon storage.

The tapes were analyzed with ROOTS ver 1.52 (Michigan State University 1994) software and programs developed in-house. Specific protocols for digitizing and analysis programs can be found in Weber (Weber 1994). Only minor modifications of the protocol were required due to changes of intervals and subsampling from the previous study. The tubes were subsampled so that only every third frame was sampled.

Root length density (RLD) was calculated by summing the lengths of roots down to a depth of 50 cm. Each tube had between 26 and 27 frames which were observed per side. The sum of the root lengths was divided by the number of frames observed and the area of a frame. The resulting number was the root length per cm^{-2}.

Two measures of root dynamics were used in this study: growth, and mortality. Because this method allows for the repeated identification and measurement of individual roots *in situ*, growth and mortality rates are potentially more accurate. In-growth methods involve removing a soil core and filling the hole with root free soil. The core would subsequently be removed and the roots within the previously root free soil would be measured to determine root growth. However, the cutting of roots and the provision of unexploited soil may increase root growth and therefore skew growth rates. Both cutting the roots and the new soil, even from the same site, may stimulate rooting and therefore not represent root growth in the steady state. Direct observation with rhizotrons and minirhizotrons are the only methods for determination of root mortality.

Root mortality and root growth were determined on an individual root basis and calculated per cm^{-2}. Root mortality was the loss of length from observation date n to observation date n+1. If the root increased in length, mortality was 0. If the root was not

observed in the second observation date, the individual root mortality was the length of the root from the first observation date. Conversely root growth was the gain in length between two dates. If the root lost length, growth was considered zero for the root. If the root initially appeared in the second date, growth was the full length of the root in the second date. In contrast to some authors (Hendrick and Pregitzer 1993*a, b*), I chose not to use color as a determinant for mortality. Previous experience has shown that roots that would have been considered dead produced new roots. Root length density would therefore be underestimated and mortality overestimated.

Field measure of carbon and nitrogen by root width and age

Three root observation chambers were placed within each site to collect individual roots of known ages. These chambers were constructed of three walls of clear acrylic with a clear acrylic bottom, a removable Masonite back panel and a plate aluminum cover (Fig. 6). The front of the chamber had two removable clear acrylic panels which allowed access to the soil and roots. Holes for the chambers were hand-dug at Hog Island and dug with large tractor driven augers and finished by hand in Konza. The chambers were then placed in the holes and the small space between the observation windows and the soil was filled with soil from the hole. The soil was gently patted in place to remove air holes along the surface of the wall. Inside the chamber the windows were covered with Styrofoam insulation and braced to add strength to the windows between tracings.

Root ages were determined along the removable window with root tracings.

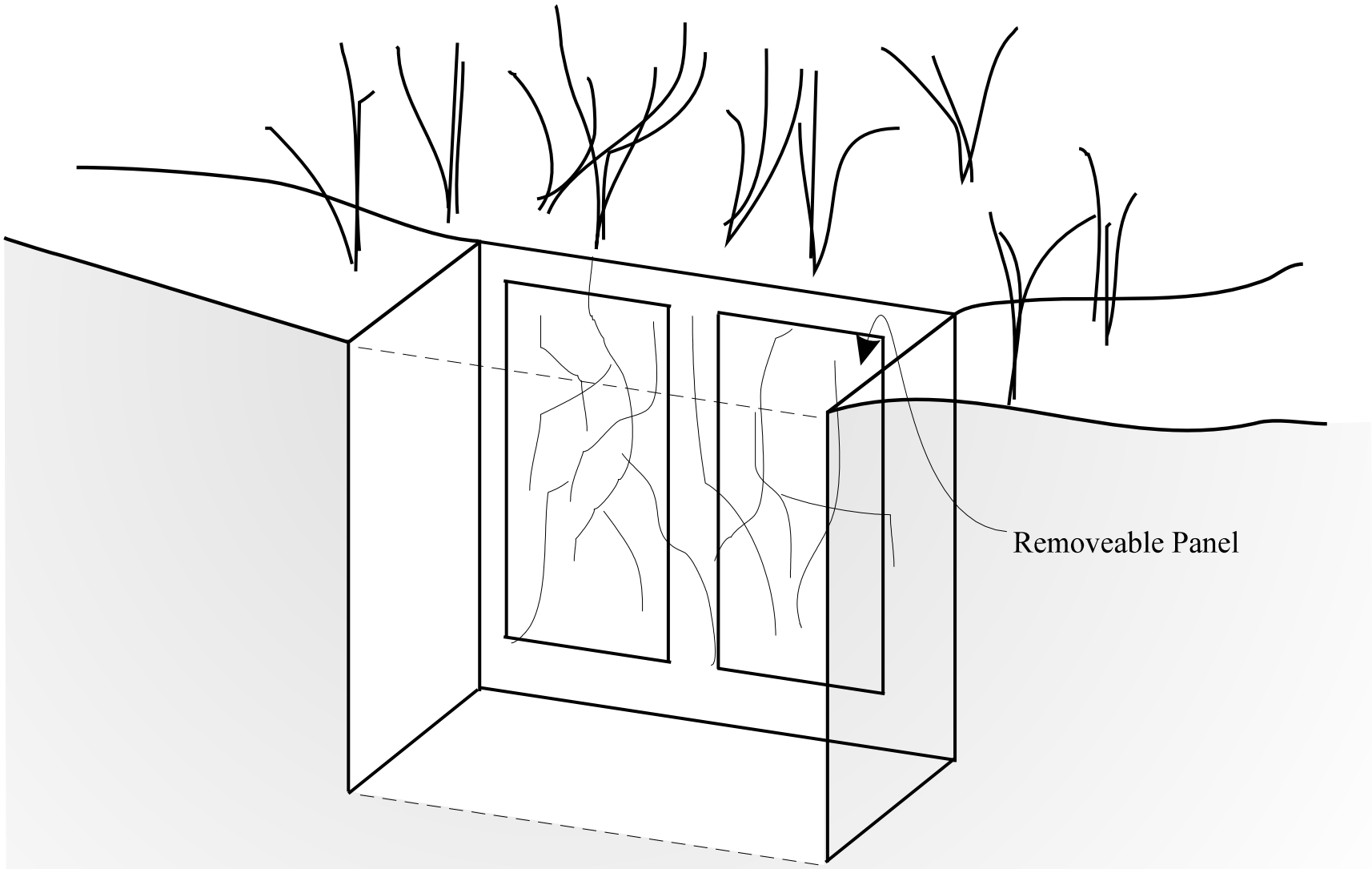

Figure 6. Buried chamber design.

Acetate sheets were placed along the surface of the removable wall and roots were traced with colored sharpie pens. The sheets were oriented on markings placed on the window from the first tracing. The tracings were made on the same dates as the minirhizotron sampling.

At the last minirhizotron sampling date, five, 5 cm sections of roots from each age class were collected. The roots were placed on dry ice and maintained at -80°C until processing. Processing consisted of gently rinsing the roots in water before measuring length with a ruler and width measurements with a radical in a dissecting scope. The roots were then oven dried at 80°C for 24 hours , weighed, and analyzed in a Carlo Erba ea1108 elemental analyzer as described above. Because of their small size, no grinding was necessary.

Greenhouse Study

A transplant experiment was performed to obtain specific physiological rates for RP and RR plants in both nitrogen rich and nitrogen poor soils. *Andropogon gerardii* (an RR plant) and *Schizachyrium scoparium* (an RP plant) were the plants selected for the study and both barrier spit dune sand (RP soil) and Konza Prairie soil (RR soil) were the selected soils. Physiological rates were obtained including photosynthesis, nitrogen uptake, and carbon allocation. Carbon flow and photosynthesis were measured by tracing ^{14}C and mass balance while nitrogen uptake was measured through mass balance.

Soils were collected for the experiment from Konza Prairie in December of 1997 and from Back Bay Wildlife Refuge (south of Virginia Beach and the Virginia barrier islands) in the summer of 1999. The soil was collected from behind a primary dune

within the Back Bay Wildlife Refuge. Both Hog Island and Back Bay Wildlife refuge soils were Newhan fine sands (Soil Conservation Service 1985). These soils are characterized as being deep, low in available nutrients, excessively drained psamments. An initial attempt of this experiment was made without exposure to ^{14}C in 1998 and the prairie soil was reused for this experiment. There was a small decline in C and N; however, the C and N contents at the end of the experiment were still at least one order of magnitude higher in the prairie soil (0.1883 ±0.0232 g N g dry mass $^{-1}$ 1.3783±0.1278 g C g dry mass $^{-1}$) than in the barrier island sand (0.0662 ± 0.0026 g N g dry mass $^{-1}$ 0.0026 ± 0.0044 g C g dry mass $^{-1}$). The mean percent nitrogen in Konza Prairie was 0.31(+-0.09), higher than the nitrogen level in the soil used for the greenhouse experiment. However the nitrogen concentration in a representative mollisol is 0.18 percent N (Brady 1990). The carbon concentration declined more precipitously from the mean of 3.72(+-1.12) on Konza Prairie. The resulting carbon content was considerably lower than the representative carbon content for mollisols (4.0 percent carbon). No carbon or nitrogen data exist immediately prior to initiation of the experiment. Unfortunately the precipitous decline of carbon and nitrogen in the Konza soil, although at the end of the study still much higher than the dune sand, reduces the difference between the soils and makes interpretation of the results more difficult.

Andropogon gerardii and *Schizachyrium scoparium* are closely related perennial grasses. Until recently these species were placed within the same genus. Rate differences should therefore reflect adaptive differences between the species rather than unselected traits.

The *Schizachyrium scoparium* was grown from one-inch sections of root stock

collected in the spring of 1999 on Hog Island. The *Andropogon gerardii* was grown from seed obtained from the USDA laboratory located adjacent to Konza Prairie. The seed was selected because of the limited selective pressure applied in the development of the seed and because the seed was both grown in the Konza Prairie area and was derived from plants from the area. The seed and rootstock were placed in sand and misted twice daily for three months. Plants were then six to eight inches tall and growth was deemed adequate for the experiment.

The pots used in the experiment were 10 inches wide, 1.5 inches deep and 18 inches long. The material used for the bottom and three of the sides was a hollocore PVC. The front of the pot consisted of a 10 inch by 18-inch sheet of clear acrylic. The front was affixed to the pot with one layer of clear tape around the edges followed by wrapping the pot with tape near the top and the bottom of the pot. In addition, duct tape was used to reinforce the packing tape on the edges of the front panel. The bottom panel had three ¼-inch holes to prevent the soil and sand from eroding out of the pots. Washed decorative quartzite pebbles filled the bottom 4 inches of each pot. Soil or sand was then added to fill each pot. Aluminum foil covered each of the pots clear fronts during the experiment to exclude light. All of the greenhouse experiment was performed in the greenhouse at Old Dominion University.

Each of the two species of grass was placed in an equal number of pots with either sand or soil resulting in a 2x2 factorial design (Fig. 7). The plants were then allowed to grow for 10 days in the pots(8/14/99 - 8/24/99) with daily watering. The plants were then removed, rinsed, patted dry, weighed, and repotted in the same pot. A sample of leaf and root was taken from each plant in the process. The samples were

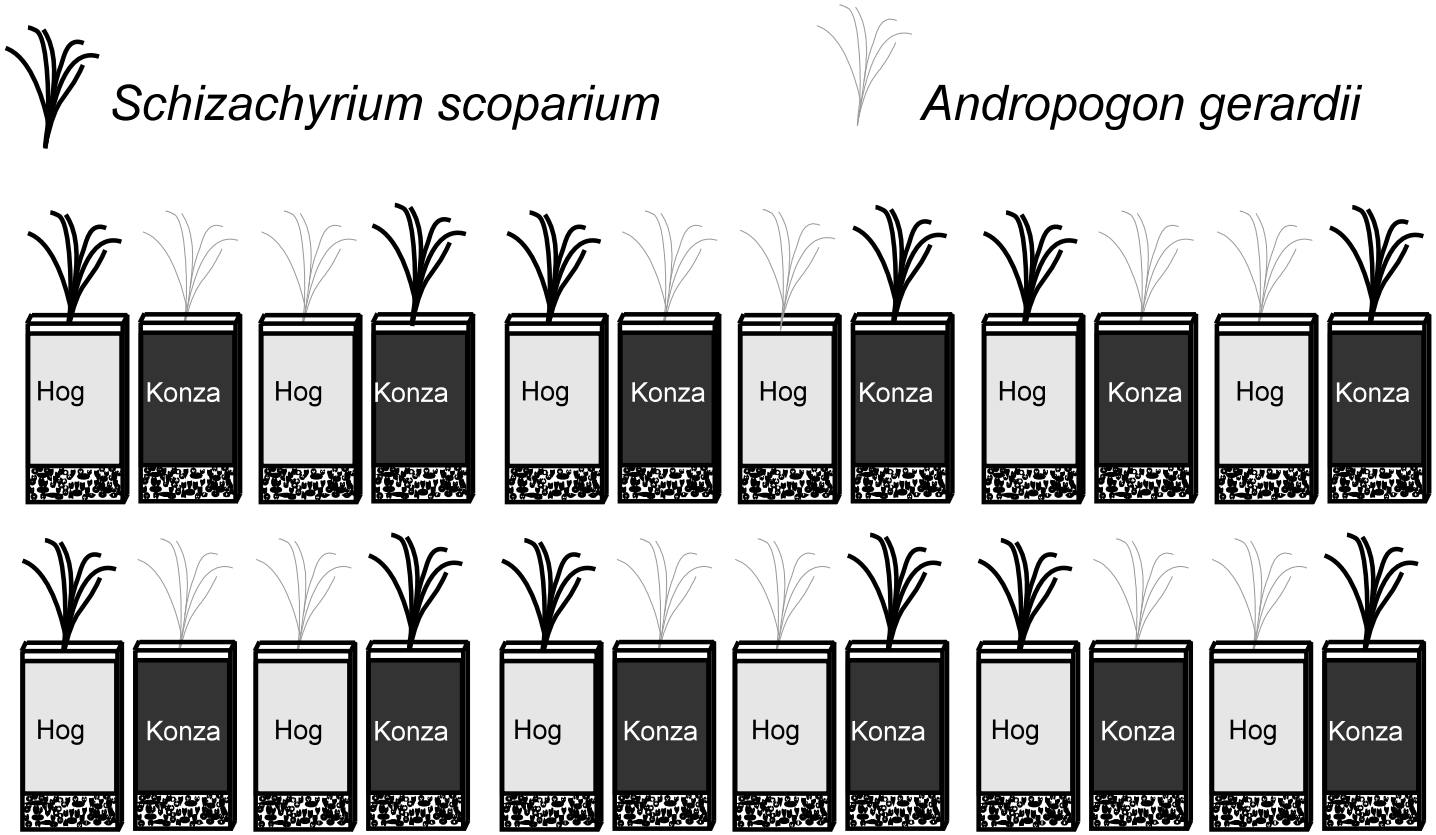

Figure 7. Design of greenhouse experiment.

oven-dried and analyzed for C and N in the carbon nitrogen analyzer. The plants were then allowed to grow for approximately two additional months with daily watering using tap water prior to exposure to ^{14}C. One month prior to sampling, the plants were exposed to continuous incandescent light to prevent flowering. Near the end of the experiment the watering system failed for approximately one and a half weeks. Consequently there were a number of plants which did not survive. All of the plants in the Konza soil survived, only five *Schizachyrium scoparium* survived in the sand and no *Andropogon gerardii* survived in the sandy soil.

At the end of the study only three experimental treatments had survived. *Schizachyrium scoparium* survived in both soil types but *Andropogon gerardii* only survived in the prairie soil. An additional change in the design included the addition of a continuous light source consisting of two incandescent light bulbs placed two feet above the plants. Two previous attempts at the experiment were foiled by flowering of the plants. Only one plant flowered at the end of the study with this slight modification to the design.

Plants were placed, in batches of 6, with an equal number of all treatments, into a plexiglass chamber to allow for ^{14}C pulse labeling of the plants on three dates (11/19/99, 11/22/99, and 11/29/99). 10 μcuries of ^{14}C were added as carbonate to a small dish of acid to evolve CO_2. The air was circulated with a biscuit fan within the chamber to ensure that there was turbulent flow throughout the chamber. Thirty minutes after release of the ^{14}C, the air was changed by forcing house air into the chamber. The gas outlets were pumped through a diffuser in NaOH solution to trap the CO_2 as carbonate. After thirty minutes the chamber was removed and the plants were sampled as described below.

After completion of the exposure to ^{14}C the plants were separated from the soil, rinsed off in DI water and patted dry with kim wipes. The tissue was then separated into above and belowground tissue and placed on dry ice to stop physiological processes. Prior to exposure to ^{14}C, any dead tissue was cut from the plants and held for nutrient analysis as detritus. The tissue was weighed wet, oven dried at 80° C for 24 hours and then weighed again to obtain dry weights. After weighing, all samples were maintained in desiccators. Coarse roots were separated after obtaining the dry weight for the combined coarse and fine root fractions. Fine root weight was total root weight minus coarse root weight.

Carbon and nitrogen values were obtained from detritus, fine roots, coarse roots, and shoots at the end of the experiment. As mentioned earlier, nitrogen and carbon values were also obtained from an individual leaf and root at the beginning of the experiment. All tissue and soil were analyzed in a Carlo Erba ea1108 elemental analyzer for carbon and nitrogen. The root and soil samples were ground with a pestle and mortar prior to analysis.

Because determining the dry weight of the roots and shoots at the beginning of the experiment would have resulted in the sacrifice of the plants, mg carbon and nitrogen belowground were calculated through a combination of the %N and %C of a root and leaf sample from each plant and the wet weight of the entire plant. Root to shoot ratios and percent water content were determined for each of the plants at the end of the experiment. The root to shoot ratios were checked to determine if there was a difference between the treatments. The same test was applied to the wet to dry weight conversion ratios. Neither of the tests were significant. This implies that at least at the end of the experiment, there

was not a difference between treatments and the conversion ratios may be stable. The specific mg of nitrogen and carbon at the start of the experiment were calculated with equations 1 through 4. The percent dry weight and root to shoot ratio were calculated individually for each plant for the N and C calculations. Change in nitrogen and carbon content was calculated by subtracting the carbon or nitrogen content at the beginning of the experiment from the content at the end of the experiment.

Eq. 1. starting leaf mg N = $PN_{LS} * P_{LE} * P_{DE} * W_{WS}$

Eq. 2. starting root mg N = $PN_{RS} * P_{RE} * P_{DE} * W_{WS}$

Eq. 3. starting leaf mg C = $PC_{LS} * P_{LE} * P_{DE} * W_{WS}$

Eq. 4. starting root mg C = $PC_{RS} * P_{RE} * P_{DE} * W_{WS}$

PN_{LS} = percent nitrogen of leaf at start of experiment
PN_{RS} = percent nitrogen of root at start of experiment
PC_{LS} = percent carbon of leaf at start of experiment
PC_{RS} = percent carbon of root at start of experiment
P_{LE} = percent leaf of plant at end of experiment
P_{RE}= percent root of plant at end of experiment
P_{DE} = percent dry weight at end of experiment
W_{WS} = wet weight of plant at start of experiment

Relative allocation belowground was calculated as both $\%^{14}C$ to the roots and increase in carbon belowground. The increase in carbon belowground was calculated as the mg carbon belowground at the start of the experiment subtracted from the mg carbon belowground at the end of the experiment divided by the total change in carbon. Change in nitrogen aboveground was similarly calculated for use in the exchange ratio calculations.

The exchange ratio is a measure of the cost of nitrogen sent aboveground in terms of carbon sent to leaves. Exchange ratios were calculated by dividing the $\%^{14}C$ sent to

the roots by the nitrogen incorporated in the leaves aboveground. This was calculated in two ways. The first was to divide the $\%^{14}C$ found in the roots by the mg of nitrogen in the leaves. This was to represent the instantaneous rate of carbon allocation. The second method was to determine the change in nitrogen aboveground divided by change in mg carbon belowground. This represented an integrated response to the environment over the duration of the experiment.

Statistics

Data were entered and manipulated in either Foxpro for the minirhizotron data or Microsoft ACCESS for all other data. Further manipulations and statistical analyses occurred in SAS ver 8.0 (1999) or SPSS (release 6.1.2, 1996).

Field costs - In addition to the variables used in the primary hypotheses (C/N ratios and biomass), several other parameters were tested in an attempt to better describe the two systems: g carbon m^{-2}, g nitrogen m^{-2}, percent C, and percent N. As described earlier, each plot consisted of a ¼ m^2 clip plot from which a core was taken. To obtain m^2 measurements, the aboveground portions were multiplied by four and the belowground portion was multiplied by the ratio of the area of the core to a m^2 (78.876).

Each variable was tested within its own splitplot ANOVA for each site. Because the Hog Island site was divided into three distinct areas, plot was nested within area. The Konza Prairie site had no such subdivisions and therefore had a simpler design (no nesting factor). PROC GLM (SAS 1999) was used for analyses. If the interaction between areas and depth was not significant, the interaction term was removed from the model to improve power.

Root observation chamber - The relationship between individual root width, weight, nitrogen, and carbon content by age was analyzed through an ANCOVA. Weight was used as a cofactor and age was a fixed effect. Width was used as a cofactor to try to determine whether function changed with width. As stated in the introduction, it is expected that nitrogen content should decline with age and carbon content should increase. This reflects a change from nutrient uptake to nutrient transport, in which case few nutrients remain within the root for long. Carbon content will likely increase with age because the roots would be used for carbon storage rather than functioning simply for support and nutrient acquisition.

Models were tested to determine the appropriate model to use with each of the parameters (Draper and Smith 1985). Each of these tests were performed by subtracting the model sums of squares between competing models and dividing by the mean square error of the model with more variables generated from PROC REG (SAS 1999). The resulting value was then compared to an F-table (Rohlf and Sokal 1981). To test whether the slopes were parallel, the full rank model (includes interaction between covariate and main effect) was compared to the ANCOVA model (no interaction term). If the full model is a significant improvement over the ANCOVA model the slopes are not parallel and a direct comparison of the means is not appropriate. If the slopes were not parallel, the full regression model will be described in the results. Particular attention was placed on the slopes and relative positions of the regression lines to each other and also to the significance of the various parameters within the model. If the full model was not a significant improvement over the ANCOVA model, a second test was run to determine whether the covariate was significant. In this case, the ANCOVA model was tested

against a model in which only the main effects were tested. If the ANCOVA model was a significant improvement, an ANCOVA model was run using PROC GLM.

The simple regression model was used when there was no effect of age on the considered variable. In a significant ANCOVA model, age is a significant factor in determining the variable. In addition, the slopes of the regression lines for each of the age classes should be equal although the intercepts will differ. The full model implies that each age class has a different relationship with width and the variable in question. In this form of model, the slopes of each age class are not equal.

Each of the selected models was carefully evaluated to determine if individual data points had undue influence on regression parameters. The studentized residual, diagonal hat matrix, Dffits and Dfbetas for each data point were evaluated for their influence using the suggested limits of Belsley et al. (1980). If data points were determined to be outside the limits in multiple measures, particularly a combination of the studentized residual (a measure of distance from the regression line), Dffits (a measure of the difference in the overall model with the removal of the point) and the Dfbetas (a measure of the change in individual slopes with removal), the point was removed from the model and the model was rerun. Data were also visually inspected by comparing the plotted position of raw data with predicted values. An iterative process of data removal was conducted in which data were removed in groups or one to three data points. The resulting model was then compared with the initial model to determine what influence removing the data had on the probability of the overall model, the adjusted R^2, and the value and significance of each model parameter.

Minirhizotrons - Increased carbon allocation belowground could be reflected in

either the density of roots or in the dynamics of root growth and mortality. The strength of the minirhizotron measurements was that it allowed for the measurement of the dynamic portion of the carbon allocation strategy. Minirhizotrons allowed for the measurement of the response to microsite enrichment. To measure the response of roots to microsite enrichment, the difference between the enriched and the unenriched sides were compared. Root mortality was compared for two differently aged cohorts of roots over the last week of the experiment (between the last two sampling dates). This was done to determine if there was a relationship between the age of a root and its response to microsite enrichment. If there was a response, one would expect that the older roots would likely die at a stable rate and the newer roots might die off at a slower rate because they were involved in nutrient uptake.

This was tested by using age class and nutrient addition as independent variables and the difference between the treated and untreated sides as the dependent variable. Age-class was coded as 0 for the older age class and 1 for the younger age class. The treatment was coded as 0, 10 and 100 times the nutrient level of the surrounding soil. A log transform of the treatment was also attempted. Root growth and RLD were similarly analyzed without the age class variable. They therefore only had two models tested, the nutrient level model and the log transformed nutrient level model. If one of the variables was significant, it improved the model and therefore significantly affected the dependent variable in question.

Greenhouse - Two factor ANOVAs were used to test differences with percent carbon, biomass, percent nitrogen, carbon-nitrogen ratio, change in carbon (growth), and ^{14}C allocation. The factors used were plant portion and treatment. The plant portion

consisted of the division of the plant into either aboveground and belowground or subdivided the belowground portion into coarse and fine roots. The treatments were the combination of the plants used and the soil in which they were planted. There were therefore three treatment groups including: *Andropogon gerardii* in Konza prairie soil, *Schizachyrium scoparium* in Konza prairie soil, and *Schizachyrium scoparium* in barrier island sand. One-way ANOVAs were used to test differences between exchange ratios. The only factor for these analyses were the same plant and soil treatments described for the other greenhouse experiment variables. Post ANOVA analyses were either REQWF post ANOVA tests for significant main effects or Tukey-Kramer multiple comparison tests when interactions were significant.

RESULTS

Field Study: Measures of Carbon and Nitrogen Costs

All analyses of the field data included both depth and a blocking factor in the ANOVA. Because of the more complicated field design on Hog Island, the blocking included both an area effect (differences between the three areas on the dune) and the individual plots within the areas. Although area*depth interactions were tested for all variables for the Hog Island data, in no case was the test significant, with many having F-values well below 1.0. Konza Prairie merely had 10 plots from which samples were obtained. Each site had four depth classes: aboveground biomass, roots found 0-10 cm belowground, roots found 10-20 cm belowground, and roots found 20-30 cm belowground. Significant differences referring to depth classes, therefore, include differences between the aboveground and belowground biomass in the system. When depth was a significant factor, a post-hoc analysis (Ryan-Einot-Gabriel-Welsch (REGW) multiple range test, (SAS 1999)) was used to determine if significant differences existed between the depth classes.

There were significant differences between depth categories for biomass on Hog Island ($p=0.0019$) (Table 6, Fig. 8) and Konza Prairie ($p=0.0001$) (Table 7, Fig. 8). The differences on Konza Prairie were large. The aboveground portion of the biomass was significantly different from the root depth classes and was more than six times the biomass of the 0-10 cm depth class of roots. Even summing the mean of the three depth classes resulted in an aboveground biomass greater than four times the total belowground biomass. The aboveground portion of Hog Island biomass was twice the root biomass. Both systems showed an insignificant decline with depth belowground.

TABLE 6 . Hog Island biomass (g m^{-2}) ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	11	29795.623	2708.693	3.40	0.0084
Area	2	2889.743	1444.871	1.82	0.1885
Plot(Area)	6	8765.323	1460.887	1.84	0.1428
Depth	3	17137.373	5712.458	7.18	0.0019
Error	20	15911.3311	795.5666		
Total	31	45706.9546			

The lack of differences between the root depth classes most likely is due to the relatively low sample number.

Neither of the systems differed significantly in percent carbon with depth (Hog Island p=0.375, Konza Prairie p=0.6032) (Tables 8,9 Fig. 9); however, there were

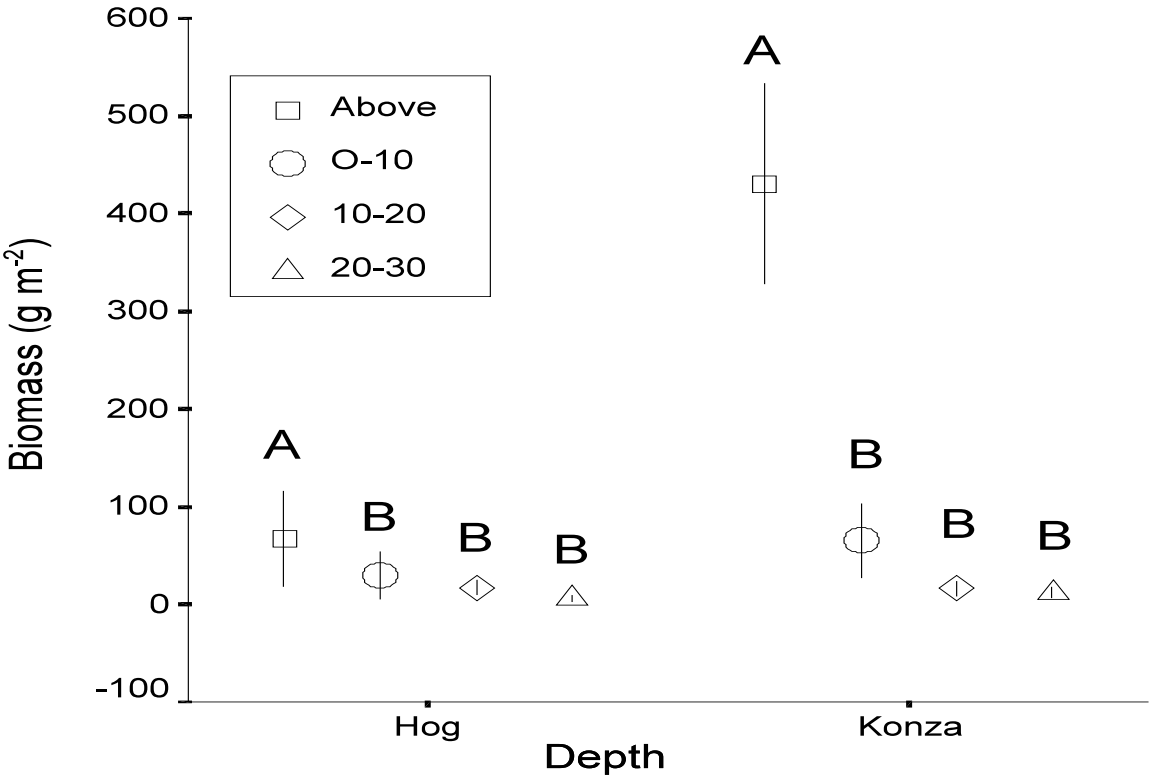

Figure 8. Mean biomass (g m^{-2}) in the field. Observations with same letter are not significantly different ($p<0.05$).

TABLE 7 . Konza Prairie biomass (g m^{-2}) ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	12	1216678.275	101389.856	14.44	0.0001
Plot	9	38989.253	4332.439	0.62	0.7709
Depth	3	1165471.340	388490.447	55.32	0.0001
Error	24	168545.352	7022.723		
Total	36	1385223.626			

significant differences in percent nitrogen on both sites (Tables 10,11). Both systems increased in the first 0-10 cm depth class from the aboveground value and then remained at the same level or declined with depth (Fig. 10). The plot and depth class factors were significant for Konza Prairie (plot p=0.0002, depth p=0.0157) (Table10) while only depth was significant on Hog Island (depth p=0.0159) (Table 11). The significant plot factor

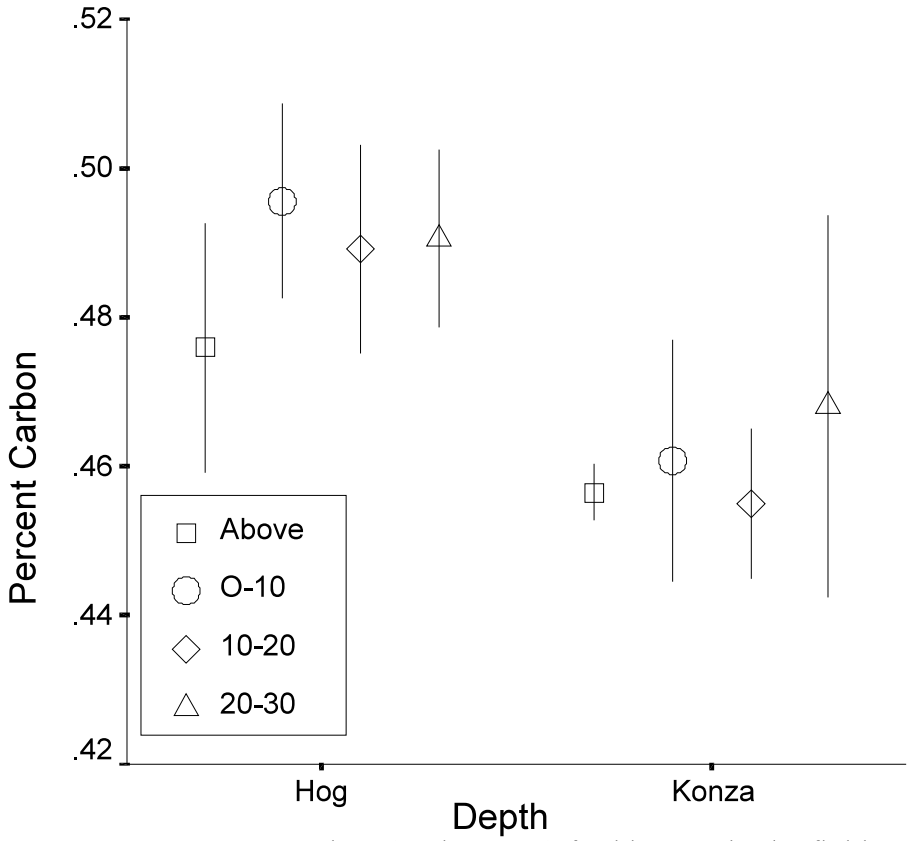

Figure 9. Mean percent carbon (% dry mass) for biomass in the field.

may represent the wider diversity found at Konza Prairie and the heterogeneous distribution of forbs which tend to have a higher concentration of nitrogen than grasses (Johnson et al. 1978). The nitrogen content of the aboveground tissue was similar to the deepest roots while the shallowest roots had the highest nitrogen content at both sites (Fig. 10).

TABLE 8. Hog Island biomass percent carbon (% dry mass) ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	11	4.1750e-03	3.7950e-04	1.06	0.4318
Area	2	1.5660e-03	7.8300e-04	2.19	0.1363
Plot(Area)	6	1.0102e-03	1.6988e-04	0.48	0.8181
Depth	3	1.3040e-03	4.3469e-04	1.22	0.3275
Error	21	7.4890e-03	3.5665e-04		
Total	31	1.1665e-02			

TABLE 9. Konza Prairie biomass percent carbon (% dry mass) ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	12	5.6954e-03	4.7462e-04	1.22	0.3246
Plot	9	4.7475e-03	5.2750e-04	1.36	0.2609
Depth	3	7.3345e-04	2.4448e-04	0.63	0.6032
Error	24	9.3240e-03	3.8850e-04		
Total	36	1.5019e-02			

TABLE 10. Konza Prairie biomass percent nitrogen (% dry mass) ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	12	1.4537e-04	1.2110e-05	5.31	0.0003
Plot	9	1.2098e-04	1.3440e-05	5.89	0.0002
Depth	3	2.8900e-05	9.6300e-06	4.22	0.0157
Error	24	5.4790e-05	2.2800e-06		
Total	36	2.0016e-04			

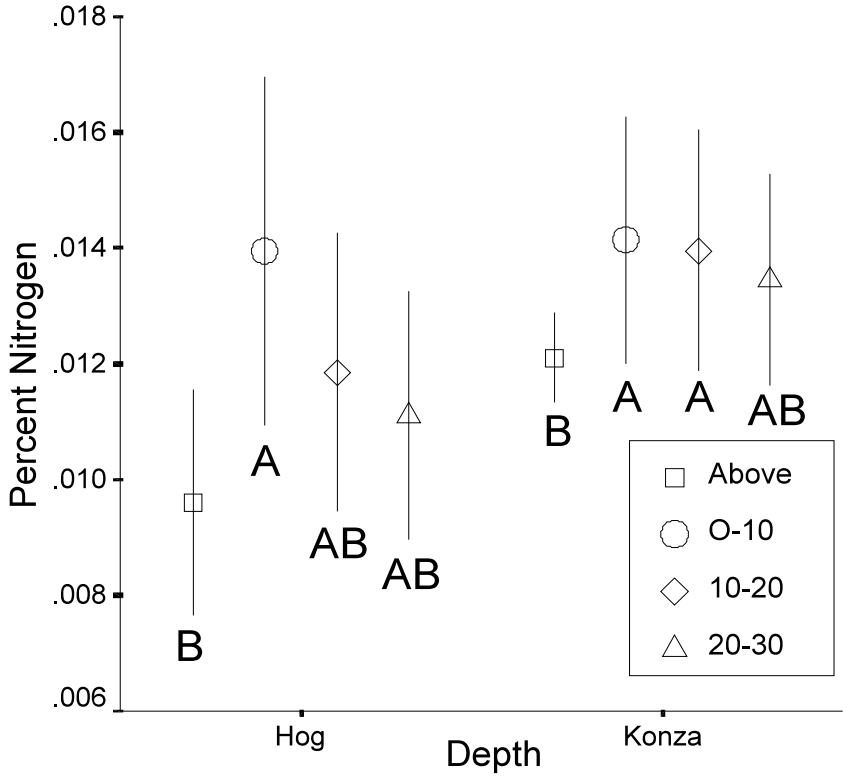

Figure 10. Mean percent nitrogen (% dry mass) for biomass in the field. Observations with the same letter are not significantly different ($p<0.05$).

TABLE 11. Hog Island biomass percent nitrogen (% dry mass) ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	11	1.5007e-04	1.3940e-05	1.99	0.0850
Area	2	8.7000e-06	4.3500e-06	0.63	0.5406
Plot(Area)	6	6.4900e-05	1.0820e-05	1.57	0.2038
Depth	3	8.9240e-05	2.9750e-05	4.33	0.0159
Error	21	1.4426e-04	6.8700e-06		
Total	32	2.9433e-04			

g N m^{-2} and g C m^{-2} were calculated by multiplying the percent carbon or nitrogen by biomass. Consequently, the large differences seen in biomass are reflected in the g N and g C m^{-2}. Because there were no significant differences in percent carbon, the g C m^{-2} values very closely reflected those found in biomass. There were significant differences in g C m^{-2} with depth on both Hog Island ($p=0.0039$) (Table 12) and on Konza Prairie

(p=0.0001) (Table 13). The aboveground live tissue g C m^{-2} was significantly higher than any root category for both sites in post hoc tests (REGW p<0.05) (Fig. 11). The mean g C m^{-2} aboveground was roughly six times the next highest depth (0-10 cm) on Konza Prairie with a more modest doubling of the carbon aboveground on Hog Island.(Fig. 11). The g C m^{-2} continued to decline with depth. There were significant differences with depth on Hog Island (p=0.0083) (Table 14) and Konza Prairie (p=0.0001) (Table 15) in g N m^{-2}. Both sites had higher g N m^{-2} aboveground in post hoc tests (REGW p<0.05), although g N m^{-2} did not significantly differ from the first 0-10 cm depth class of roots on Hog Island (Fig. 12). There was a similar pattern in g N m^{-2} to that found in biomass and g C m^{-2}. Both sites had significantly higher quantities of nitrogen aboveground than belowground although the distinction was less clear on Hog Island where the first root depth class (0-10 cm) was contained within both a group composed exclusively of belowground tissue and a group including the aboveground tissue and the 0-10 cm depth class. Both Hog Island and Konza Prairie had an insignificant decline in g N m^{-2} with depth (Fig. 12).

TABLE 12. Hog Island g C m^{-2} ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	11	7309.7326	664.5212	3.07	0.0142
Area	2	772.2632	386.132	1.78	0.1935
Plot(Area)	6	2314.133	385.689	1.78	0.1539
Depth	3	3992.473	1330.824	6.15	0.0039
Error	20	4326.9703	216.3485		
Total	31	11636.703			

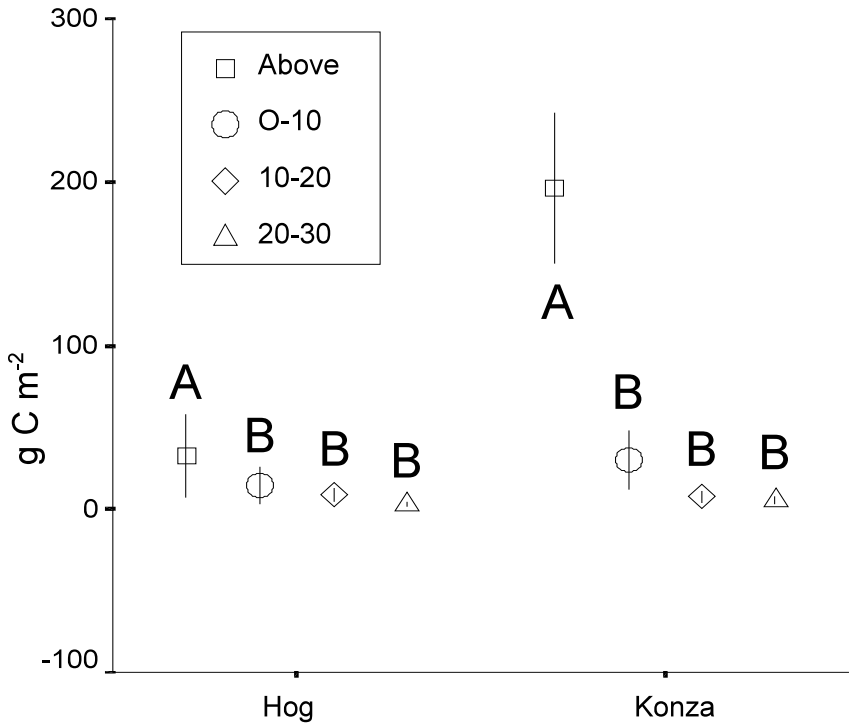

Figure 11. Mean g C m^{-2} in the field. Observations with the same letter are not significantly different ($p<0.05$).

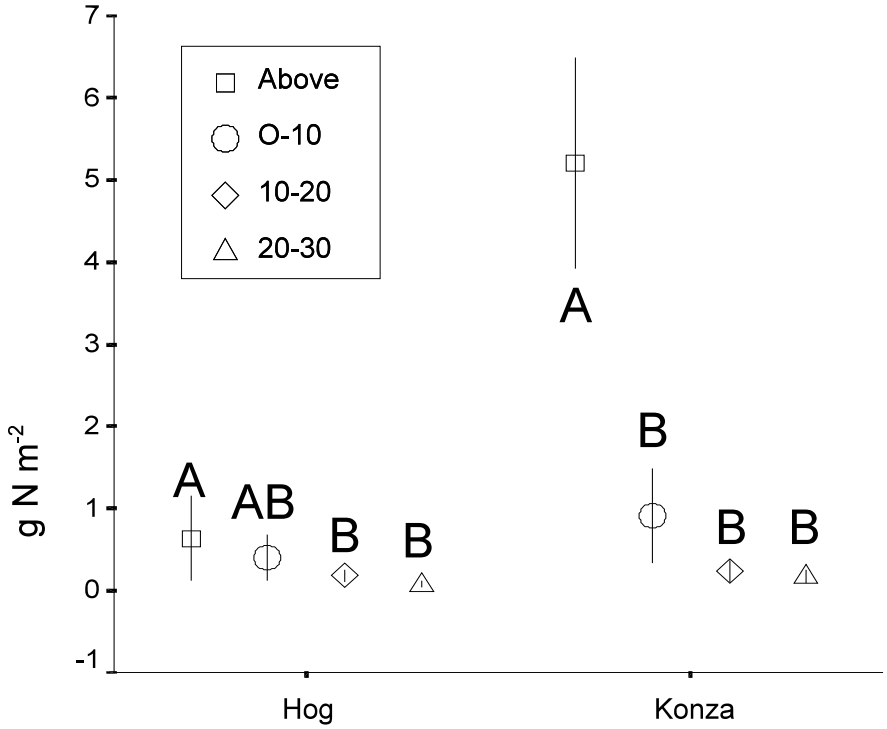

Figure 12. Mean g N m^{-2} in the field. Observations with the same letter are not significantly different ($p<0.05$).

TABLE 13. Konza Prairie g C m^{-2} ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	12	252075.311	21006.276	14.84	0.0001
Plot	9	7613.383	845.932	0.60	0.7863
Depth	3	241617.767	80539.256	56.90	0.0001
Error	24	33970.510	1415.438		
Total	36	286045.821			

TABLE 14. Hog Island g N m^{-2} ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	11	2.7813	0.253	2.67	0.0271
Area	2	0.355	0.177	1.88	0.1792
Plot(Area)	6	0.844	0.141	1.49	0.2328
Depth	3	1.467	0.489	5.17	0.0083
Error	20	1.892	0.095		
Total	31	4.673			

TABLE 15. Konza Prairie g N m^{-2} ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	12	175.534	14.628	12.77	0.0001
Plot	9	6.430	0.714	0.62	0.7656
Depth	3	166.957	55.652	48.57	0.0001
Error	24	27.501	1.146		
Total	36	203.034			

Carbon to nitrogen ratio was significantly different with depth for both Hog Island (p=0.0082) (Table 16) and Konza Prairie (0.0425) (Table 17). Hog Island declined from the high in the aboveground tissue and then rose with depth (Fig. 13). After a similar, if less dramatic, decline in the Konza Prairie carbon-nitrogen ratio from the aboveground to the first root depth class, the carbon-nitrogen ratio increased to levels near those from the aboveground tissue. Because percent carbon was not significant, percent nitrogen likely

drives the differences in the carbon nitrogen ratios. This also is reflected in the significant plot factor in the ANOVA table for Konza Prairie (p=0.0009) (Table 17).

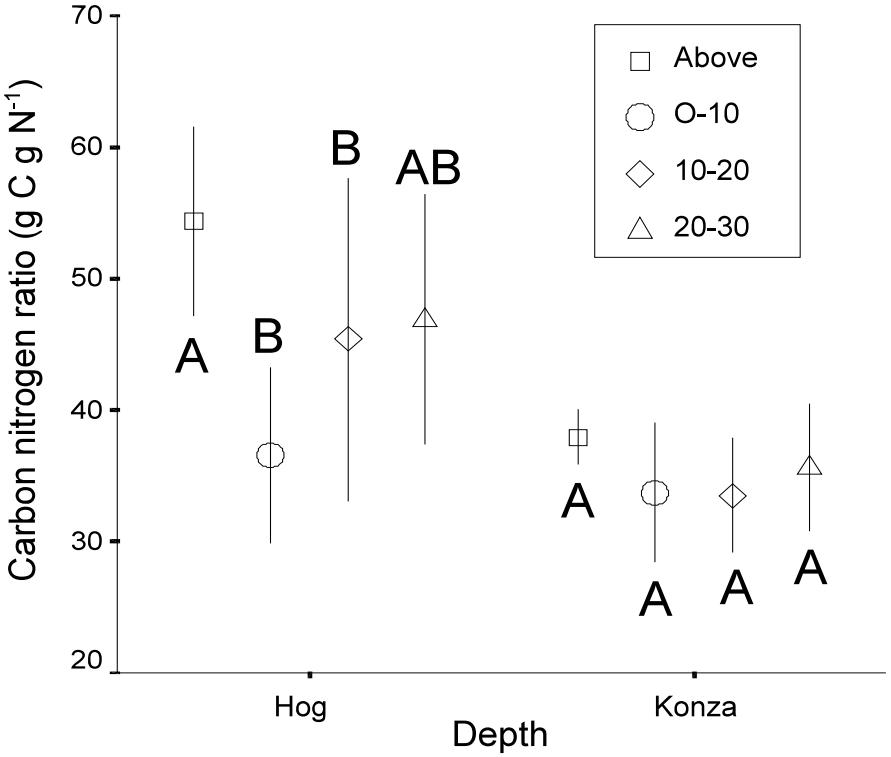

Figure 13. Mean C/N in the field. Observations with the same letter are not significantly different (p<0.05).

TABLE 16. Hog Island carbon-nitrogen ratio ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	11	2288.701	208.064	2.34	0.0473
Area	2	424.408	212.204	2.39	0.1174
Plot(Area)	6	596.256	99.376	1.12	0.3867
Depth	3	1380.360	460.120	5.18	0.0082
Error	20	1776.518	88.256		
Total	31	4065.219			

TABLE 17. Konza Prairie carbon-nitrogen ratio ANOVA.

Source	DF	SS	MS	F value	Pr>F
Model	12	795.768	66.314	4.30	0.0012
Plot	9	675.107	75.012	4.86	0.0009
Depth	3	146.986	48.995	3.17	0.0425
Error	24	370.524	15.439		
Total	36	1166.291			

Field Study: Root Observation Chambers

Differences of cost between ages and widths of roots were assessed using a variety of models. Width was a continuous factor and age class was a fixed factor throughout the analyses. The models included a simple regression model, an ANCOVA and a full regression model. In the simple regression model, only width was included. If this model fit the data best, age did not add any information to help determine the dependent variable. The ANCOVA model adds the age class as a variable to the model. If this shows the best fit, age is an important factor. A T-test of the width parameter indicates its significance in the ANCOVA. If the full model is chosen, both the slopes and the y-intercept may be different. This implies a more complex relationship with both age and width. For both the ANCOVA and the full model, both the R^2 and the p-value

are important for interpretation. A significant p-value simply implies that the regression is significantly different from a horizontal line. In other words, there is a change in the dependent variable with width. The R^2 gives a measure of fit. If the R^2 is low, even if there is a significant p-value, the individual values do not match the regression line well.

There were no significant differences in percent carbon between individual roots (Hog Island p=0.927, Konza Prairie p=0.067) (Table 18, Fig. 14). This was also true for the field data. The adjusted R^2 was extremely low for both systems (Hog Island adj-R^2=-0.030, Konza Prairie adj-R^2=0.063). There were therefore no significant differences by width or age.

The ANCOVA model was the model that best described the data for percent nitrogen in both Hog Island and Konza Prairie. Although the ANCOVA model was the best model for Konza Prairie, there was not a significant relationship between the variables and percent nitrogen (p=0.069) (Table 18). Percent nitrogen declined with both age and width for Hog Island (Fig. 15). However, there was a relatively low adjusted R^2 (adj-R^2=0.373) (Table 18). Age and width increased with all other variables.

Both age and width were significant factors for carbon-nitrogen ratios at Hog Island and Konza Prairie. An ANCOVA model was the most appropriate model for this variable in both systems (Table 18). Age and width were extremely good predictors of carbon-nitrogen ratio (adj-R^2 = 0.912). There was also a relatively strong relationship between age and width with carbon-nitrogen ratio for Konza Prairie (adj-R^2=0.548). For both systems, the carbon nitrogen ratio increased both as roots age and with width (Fig. 16).

Table 18. Model selection results for root observation chambers. Variables listed are dependent variables; independent variables included width as a covariate and age as a fixed effect. Italicized models were the chosen model. Bold F-values were significant at the 0.05 level. Site H =Hog Island, K=Konza Prairie. R^2s are adjusted R^2s.

		Full Model				ANCOVA				Simple Model				model comp. F	
Variable	Site	SS	MS	p-value	R^2	SS	MS	p-value	R^2	SS	MS	p-value	R^2	full/anc	anc/simp
% C	H	0.00415	0.00667	0.986	-0.148	0.00406	0.00624	0.884	-0.074	*0.00005*	*0.00598*	*0.927*	*-0.030*	0.013	0.643
	K	0.03360	0.00562	0.337	0.024	0.02238	0.00562	0.281	0.025	*0.0192*	*0.0054*	*0.067*	*0.063*	1.996	0.566
% N	H	0.06643	0.00250	0.001	0.388	*0.5951*	*0.00256*	*0.001*	*0.373*	0.05043	0.00268	0.000	0.344	2.768	**3.547**
	K	0.03479	0.00406	0.159	0.088	*0.03064*	*0.00394*	*0.069*	*0.114*	0.01586	0.00413	0.058	0.071	1.022	**3.751**
g C mm^{-1}	H	*0.71631*	*0.00078*	*0.000*	*0.964*	0.65742	0.00263	0.000	0.879	0.63576	0.00313	0.000	0.856	**75.500**	**8.236**
	K	1.42882	0.01917	0.000	0.647	*1.42131*	*0.01829*	*0.000*	*0.663*	1.31337	0.02022	0.000	0.627	0.392	**5.902**
g N mm^{-1}	H	*0.00004*	*2.787e-6*	*0.031*	*0.218*	0.000028	3.01e-6	0.041	0.156	0.00003	2.85e-6	0.004	0.200	**4.462**	-0.686
	K	0.00032	0.00012	0.018	0.230	0.00031	0.00002	0.004	0.265	*0.0003*	*0.00002*	*0.000*	*0.300*	0.083	0.500
C/N	H	10008.97000	29.395	0.000	0.978	*9986.587*	*28.221*	*0.000*	*0.912*	9601.8	38.171	0.000	0.881	0.762	**13.635**
	K	7861.66000	156.869	0.000	0.549	*7536.22*	*157.2134*	*0.000*	*0.548*	6123.167	187.730	0.000	0.461	2.062	**8.988**

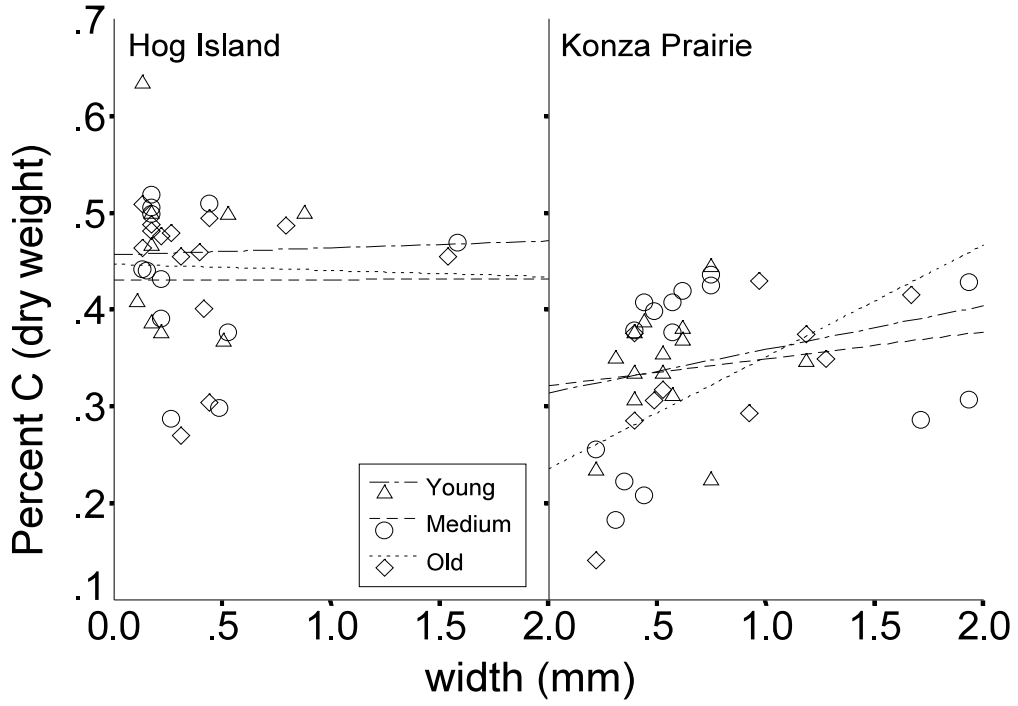

Figure 14. Scatter plot with regressions for buried chamber percent carbon. Lines represent regressions for each age class. The R^2 of regressions for individual age classes for Hog Island were: Old =0.0011, Medium=0.0000, Young=0.0005. The R^2 of regressions for individual age classes at Konza Prairie were: Old =0.4395, Medium=0.0333, Young=0.0342.

The highest adjusted R^2 of any of the variables for either system was ng C mm^{-1} (Hog Island adj-R^2=0.964, Konza Prairie adj-R2=0.663) (Table 18). The full model was chosen for Hog Island indicating that there were different slopes for the different age classes, while the ANCOVA model was chosen for Konza Prairie. The ng C mm^{-1} increased the most in the oldest roots and increased the least in the youngest roots (Fig. 17). At both Hog Island and Konza Prairie, the oldest roots had the highest relative ng C mm^{-1} and the youngest roots had the lowest relative ng C mm^{-1}. The ng C mm^{-1} also increased with width.

Both systems ng N mm^{-1} increased with width (Fig. 18). The oldest roots also had

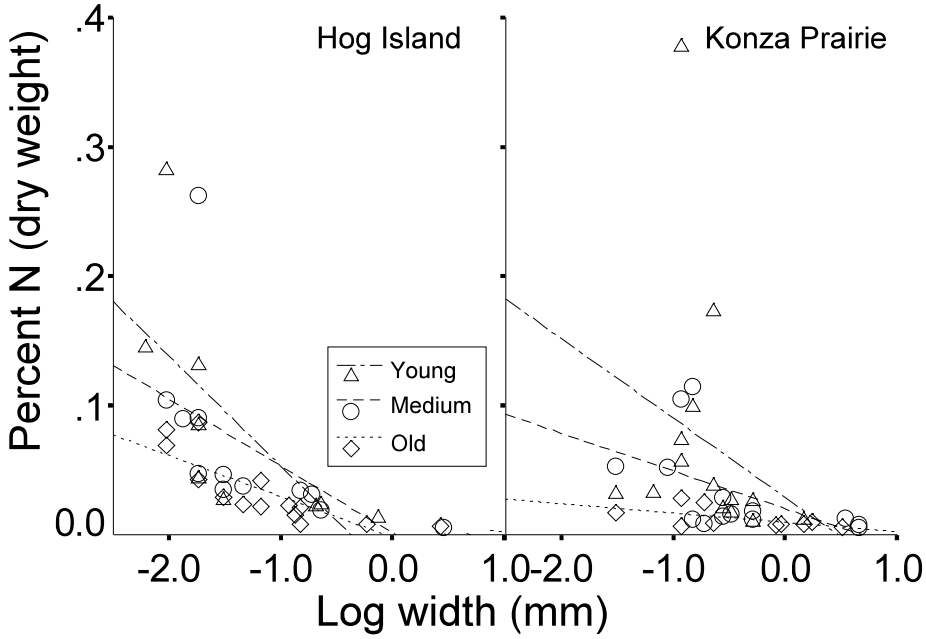

Figure 15. Scatter plot with regressions for buried chamber percent nitrogen. Lines represent regressions for each age class. The R^2 of regressions for individual age classes for Hog Island were: Old =0.6502, Medium=0.2918, Young=0.4740. The R^2 of regressions for individual age classes at Konza Prairie were: Old =0.3319, Medium=0.2841, Young=0.0675.

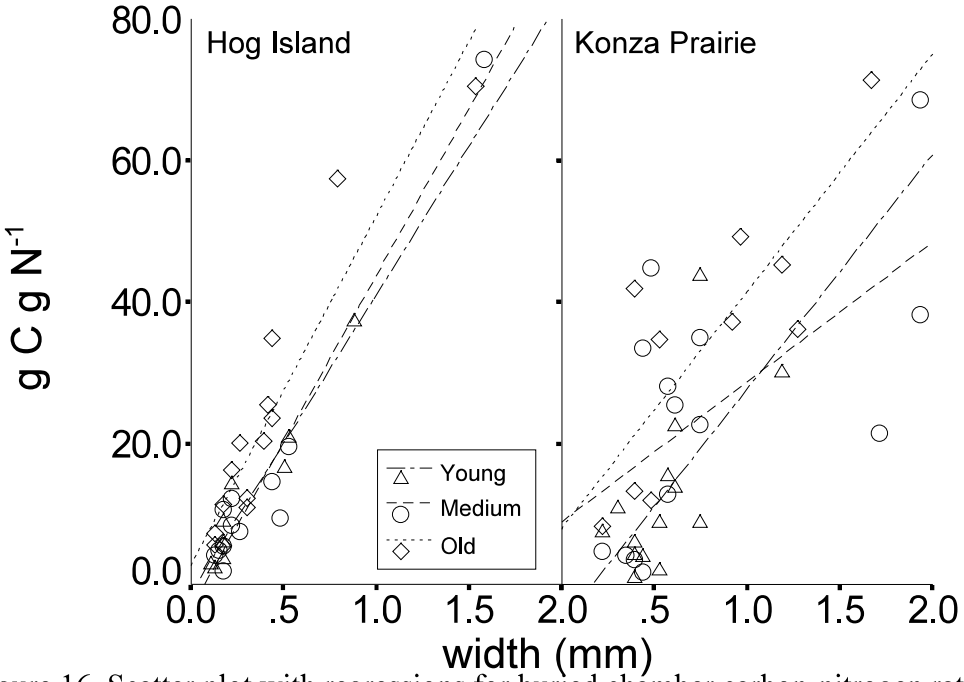

Figure 16. Scatter plot with regressions for buried chamber carbon-nitrogen ratio. Lines represent regressions for each age class. The R^2 of regressions for individual age classes for Hog Island were: Old =0.8805, Medium=0.9589, Young=0.9318. The R^2 of regressions for individual age classes at Konza Prairie were: Old =0.6684, Medium=0.3888, Young=0.4403.

the steepest slope at Hog Island and the youngest had the flattest slope. The higher order models were not a significant improvement over the simple regression model for Konza Prairie. Age, therefore, is not an important factor for determining ng N mm^{-1} at Konza Prairie. The adj-R^2 for width was moderately low (adj-R^2=0.300), however, indicating a relatively weak relationship with width as well. Although the full model theoretically best described the relationship between variables for Hog Island, no t-tests other than that for the intercept and width were significant ($p<0.05$) for the individual variables. The adjusted R^2 for the simple model was 0.200, marginally worse than that for the full model (0.218). Because of the marginal improvement of the fit with the full model, the differences with age in the model should be interpreted cautiously.

Greenhouse Experiment

The purpose of this study was to determine the costs and benefits associated with the roots of an RR plant and an RP plant. The costs are described in terms of carbon and nitrogen as well as the ratio of carbon to nitrogen in the aboveground biomass, coarse roots and fine roots. The benefit of allocating resources as well as the costs were determined by both an analysis of carbon partitioning and growth. Finally, the exchange ratios of carbon to the roots for nitrogen in the aboveground biomass are discussed. As mentioned in the methods section, there were three treatments in this study: *Andropogon regardii* (RR plant) in Konza Prairie soil, *Schizachyrium scoparium* (RP plant) in Konza Prairie soil, and *Schizachyrium scoparium* (RP plant) in dune sand.

Although the portion of the plant was a significant factor for percent carbon ($p=0.0001$), treatment was not significant ($p=0.3535$) (Table 19). The percent carbon

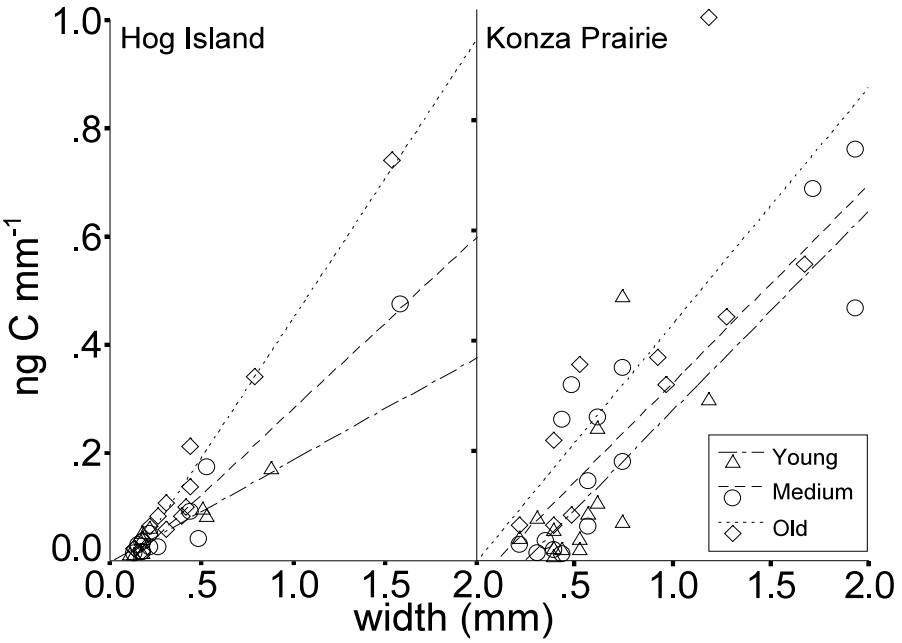

Figure 17. Scatter plot with regressions for buried chamber carbon per mm root. Lines represent regressions for each age class. The R^2 for individual age classes for Hog Island were: Old =0.9754, Medium=0.9514, Young=0.9256. The R^2 for individual age classes at Konza Prairie were: Old =0.5348, Medium=0.7881, Young=0.4135.

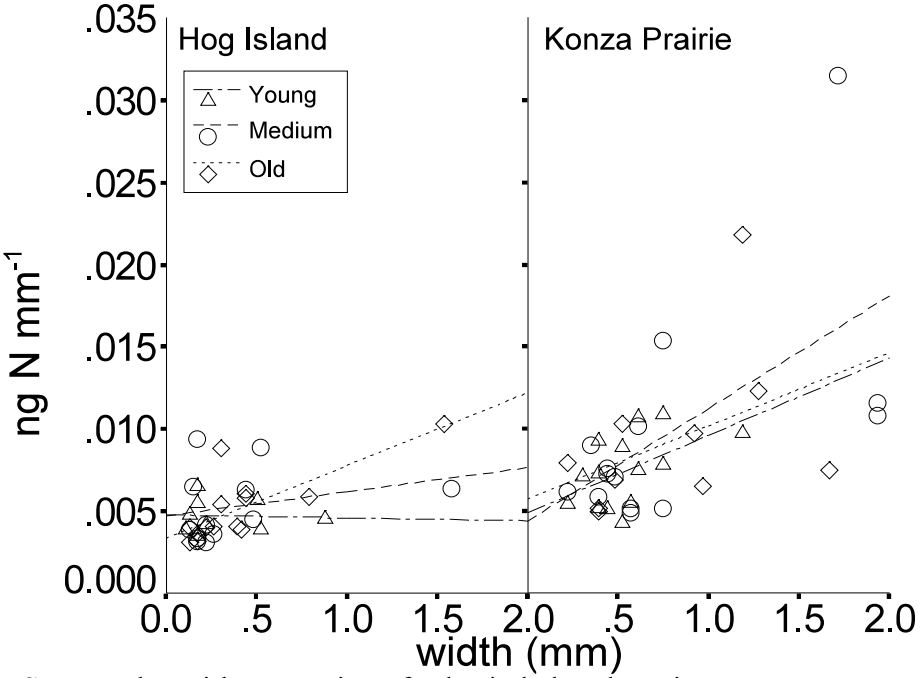

Figure 18. Scatter plot with regressions for buried chamber nitrogen per mm root. Lines represent regressions for each age class. The R^2 for individual age classes for Hog Island were: Old =0.5936, Medium=0.0722, Young=0.0021. The R^2 for individual age classes at Konza Prairie were: Old =0.1812, Medium=0.3498, Young=0.2662.